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(54) Title: HETERODUPLEX TRACKING ASSAY (HTA) FOR GENOTYPING HCV			
(57) Abstract			
<p>A heteroduplex tracking assay (HTA), a hybridization based method of determining the genetic relationship between two or more viral genomes, for genotyping HCV is disclosed. The HTA for genotyping HCV was developed using single stranded probes derived from the carboxyl terminus of core and part of the E1 for HCV subtypes (1a, 1b, 2a, 2b, and 3a). HTA is more accurate than RFLP for sub-typing HCV and has potential for identifying new variants and is useful for epidemiological studies.</p>			

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HETERODUPLEX TRACKING ASSAY (HTA) FOR GENOTYPING HCV**Field of the Invention**

This invention relates to genotyping hepatitis C viruses (HCV). In particular, this
5 invention relates to specific primers preferably from the core and envelope region of
HCV and a method to determine genotypes of HCV with a heteroduplex mobility or
tracking assay which, in turn, utilizes specific primers.

Background of the Invention

10 Viral hepatitis is known to be caused by five different viruses known as hepatitis
A, B, C, D, and E. HAV is an RNA virus and does not lead to long-term clinical
symptoms. HBV is a DNA virus. HDV is a dependent virus that is unable to infect cells
in the absence of HBV. HEV is a water-borne virus. HCV was first identified and
characterized as a cause of non-A, non-B hepatitis NANBH. (Houghton et al., EPO Pub.
15 Nos. 388,232 and 318,216). This led to the disclosure of a number of general and
specific polypeptides useful as immunological reagents in identifying HCV. See, e.g.,
Choo et al. (1989) Science, 244:359-262; Kuo et al., (1989) Science 244:362-364 and
Houghton et al, (1991) Hepatology 14:381-388.

10 HCV is a single stranded RNA virus, distantly related to the pestivirus and
flavivirus and it is the causative agent of the vast majority of transfusion-associated
hepatitis and of most cases of community-acquired non-A, non-B hepatitis worldwide.
The HCV genome consists of 5' and 3' noncoding (NC) regions that flank a single long
open reading frame (ORF). This ORF encodes for three structural proteins at the amino-
terminal end and for six nonstructural (NS) proteins at the carboxyl-terminal end. The
25 structural proteins are represented from the nucleocapsid (core; C) proteins and two
glycoproteins, envelope 1 (E1) and envelope 2 (E2). The nonstructural proteins are
named NS2, NS3, NS4a, NS4b, NS5a, NS5b. The 5'NCR is the most highly conserved
part of the HCV genome, whereas the sequence of the two envelope proteins (E1 and E2)
is highly variable among different HCV isolates. The highest degree of variation has
30 been observed in a region within E2, now commonly termed hypervariable region 1

(HVR1) or E2HV. A second variable region called the HVR2 also exists in a subset of isolates. Typically, the genetic heterogeneity of HCV has been classified under two headings quasispecies and genotypes. As used herein the term "quasispecies" refers to the genetic heterogeneity of the HCV population within an infected individual. As used 5 herein the terms "genotype" and "subtype" refer to the genome heterogeneity observed among different HCV isolates. The analysis of nucleic acid sequence variation of the HCV genome, a positive stranded of approximately 9.4 kb RNA molecule, suggest that genetic variability is associated with important virological and clinical implications.

The prototype isolate of HCV was characterized in EP Publications Nos. 318,216 10 and 388,232. As used herein, the term "HCV" includes newly isolated NANBH viral species. The term "HCV-1" refers to the virus described in the above-mentioned publications.

Since the initial identification of HCV, at least 6 different major viral types have 15 been identified (full length genomes reported) and designated Type 1, 2, 3, 4, 5 and 6. Within these types are numerous subtypes. The type of virus with which a patient is infected may affect the clinical prognosis and also response to various treatments. See, 20 Yoshioka et al., (1992) Hepatology 16:293-299. Considering that the most serious clinical outcome of HCV infection is hepatocellular carcinoma, it would be useful to be able to determine with which type or types of HCV a patient is infected. It is thus of particular importance to develop an, accurate, reliable assay for HCV genotyping and subtyping, that, without requiring the sequencing, could also give the genetic divergence intra-subtype. Several classification have been proposed for HCV genotyping based on analysis of different regions, because the ideal nucleotide sequence-based system, using the complete viral genome is not practical.

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Summary of the Invention

The present invention includes primers and methods for the characterization of 30 HCV genotyping and of variation intra-subtype based on the heteroduplex tracking assay (HTA). The preferred probes/primers were single stranded derived from the carboxyl terminus of core and part of the E1 region of HCV.

The HTA is a hybridization based method of determining the genetic relationship between two or more viral genomes. The basis of the method is that related DNA products coamplified from divergent templates reanneal randomly to form heteroduplexes that migrate with reduced mobility in systems designed to separate molecules on the basis of size such as neutral polyacrylamide gels. HTA was originally used to genotype HIV-1 and to follow the *in vivo* evolution of HIV-1 in patients and populations. See, e.g., Delwart et al., (1993) *Science* 262:1757-1761 and Delwart et al., (1994) *J. Virol.* 68:6772-6883.

One aspect of the invention is a method for genotyping HCV comprising the steps of denaturing and reannealing partially complementary DNA or RNA strands and detecting sequence variation by noting electrophoretic mobility of the DNA heteroduplexes on a system designed to separate molecule on the basis of size such as by following electrophoresis through a polyacrylamide or MDE gel.

Another aspect of the invention relates to the probes used in the genotyping which were selected from the core and E1 region of the HCV genome.

Another aspect of the invention relates to a method of predicting the response to drug therapy of a patient infected with a strain of HCV by determining the sensitivity of different known genotypes to drug therapy, determining the genotype of the HCV strain infecting the patient and comparing the genotype with its drug therapy sensitivity to predict the patient's response to the drug therapy.

Another aspect of the invention relates to therapeutic vaccines and predicting which therapeutic vaccine should be utilized by determining the genotype of a patient infected with a strain of HCV and administering a therapeutic vaccine of the same genotype.

Another aspect of the invention relates to prophylactic vaccines and predicting which vaccine should be administered to a certain population sample by determining the prevalent genotypes in a like sample and administering a prophylactic vaccines of a genotype likely to be the prevalent genotype to the population sample.

Another aspect of the invention relates to the ability to discovering new genotypes of HCV using the method of the invention.

Brief Description of the Figures

Figures 1A - 1E are autoradiograms showing homoduplexes and heteroduplexes of the samples to be typed with the probes of known genotypes (ss probes are of genotypes 1a, 1b, 2a, 2b, 3a in Figs. 1A- 1E respectively, lane on far left of MDE gel).
5 The homoduplex (h) (ss probe to the double stranded RT-PCR product of known genotype ptp from which it was derived) is shown adjacent to the probe. The heteroduplexes of the RT-PCR products from the 15 dialysis patients (nos. 1, 2, 3, 4, 7, 18, 20, 22, 23, 24, 26, 28, 30, 33, 35) hybridized to the ss probe is designated above the 10 appropriate lane in each Figure.

Figures 2A - 2C are dendograms, i.e., phylogenetic trees showing the relatedness of each partial E1 nucleotide sequence, formed by comparing partial E1 sequences obtained by sequencing of putative type 1 (nt 625-930), type 2 (nt 583-915) or type 3 (nt 558-834) isolates from the dialysis patients described hereinto published genotype 15 sequences for type 1a (HCV-1) (Choo, et al, PNAS (1991) 88:2451-2455, all nucleotide, "nt", designations according to this paper), 1b (HCV-J) (Kato et al, PNAS (1990) 87:9524-2528), 2a (HC-J6) (Okamoto et al Virol. (1992) 188:331-341), 2b (HC-J8) (Okamoto et al Virol. (1992) 188:331-341), 2c (Bukh, et al PNAS (1993) 90:8234-8239) and 3a (NZL-1) (Sakamoto, et al) J. Gen. Virol. (1994) 75:1761-1768 over the same 20 region of the genome.

Figure 2D is a dendogram, phylogenetic tree, formed by comparing either partial 5'UTR sequences of isolates 23, 30 and 33 obtained by direct sequencing with published type 1, 2 and 3 (nt -274 to -81) genotype sequences for the same region of the genome.

Figures 3A - 3D show the nucleotide sequences for dendograms depicted in 25 Figures 2A - 2D.

Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, 30 polypeptide and nucleic acid synthesis, and immunology, which are within the skill of

the art. Such techniques are explained fully in the literature. See e.g., Sambrook, et al., MOLECULAR CLONING: A LABORATORY MANUAL, SECOND EDITION (1989); DNA CLONING, VOLUMES I AND II (D.N. Glover ed. 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed. 1984); NUCLEIC ACID HYBRIDIZATION (B.D. Hames & S.J. Higgins eds. 1984); TRANSCRIPTION AND TRANSLATION (B.D. Hames & S.J. Higgins eds. 1984); ANIMAL CELL CULTURE (R.I. Freshney ed. 1986); IMMOBILIZED CELLS AND ENZYMES (IRL Press, 1986); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984); the series, METHODS IN ENZYMOLOGY (Academic Press, Inc.); GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively), Mayer and Walker, eds. (1987), IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification. All publications, patents, and patent applications cited herein are incorporated by reference.

The term "recombinant polynucleotide" as used herein intends a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation:

(1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide other than that to which it is linked in nature, or (3) does not occur in nature.

The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA and RNA. It also includes known types of modifications, for

example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

By "PCR" is meant herein the polymerase chain reaction (PCR) technique, disclosed by Mullis in U.S. Pat. Nos. 4,683,195 (Mullis et al) and 4,683,202, incorporated herein by reference. In the PCR technique, short oligonucleotide primers are prepared which match opposite ends of a desired sequence. The sequence between the primers need not be known. A sample of DNA (or RNA) is extracted and denatured (preferably by heat). Then, oligonucleotide primers are added in molar excess, along with dNTPs and a polymerase (preferably Taq polymerase, which is stable to heat). The DNA is replicated, then again denatured. This results in two "long products," which begin with the respective primers, and the two original strands (per duplex DNA molecule). The reaction mixture is then returned to polymerizing conditions (e.g., by lowering the temperature, inactivating a denaturing agent, or adding more polymerase), and a second cycle initiated. The second cycle provides the two original strands, the two long products from cycle 1, two new long products (replicated from the original strands), and two "short products" replicated from the long products. The short products have the sequence of the target sequence (sense or antisense) with a primer at each end. On each additional cycle, an additional two long products are produced, and a number of short products equal to the number of long and short products remaining at the end of the previous cycle. Thus, the number of short products grows exponentially with each cycle. This amplification of a specific analyte sequence allows the detection of extremely small quantities of DNA.

The term "3SR" as used herein refers to a method of target nucleic acid amplification also known as the "self-sustained-sequence-replication" system as described in European Patent Publication No. 373,960 (published June 20, 1990).

5 The term "LCR" as used herein refers to a method of target nucleic acid amplification also known as the "ligase chain reaction" as described by Barany, Proc. Natl. Acad. Sci. (USA) (1991) 88:189-193.

An "open reading frame" (ORF) is a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

10 A "coding sequence" is a polynucleotide sequence which is translated into a polypeptide, usually via mRNA, when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to, cDNA, and recombinant polynucleotide sequences.

15 As used herein, the term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, 20 polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

25 A polypeptide or amino acid sequence "derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 3-5 amino acids, and more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence. This terminology also includes a polypeptide expressed from a designated nucleic acid sequence.

The protein may be used for producing antibodies, either monoclonal or polyclonal, specific to the protein. The methods for producing these antibodies are known in the art.

"Recombinant host cells", "host cells," "cells," "cell cultures," and other such terms denote, for example, microorganisms, insect cells, and mammalian cells, that can be, or have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transformed. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. Examples for mammalian host cells include Chinese hamster ovary (CHO) and monkey kidney (COS) cells.

By "cDNA" is meant a complimentary mRNA sequence that hybridizes to a complimentary strand of mRNA.

By "purified" and "isolated" is meant, when referring to a polypeptide or nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000, can be present).

By "pharmaceutical acceptable carrier," is meant any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers; and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

The therapeutic compositions typically will contain pharmaceutically acceptable vehicles, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect.

5 Evidence indicates that different HCV genotypes may have different pathogenicities as well as distinct geographical distributions and may elicit partly different serological profiles in infected patients. See Cammarota, et al. *J. Clin. Microb.* (1995) 33:2781-2784. The invention includes methods for detecting HCV and identifying infection by different types of HCV. The invention includes genotyping 10 HCV, the potential to discover a new genotype of HCV, and assessing viral populations for ability to predict response to drug therapy. The invention also includes probes for use in the genotyping of HCV.

The methods for genotyping HCV include but are not limited to a heteroduplex tracking or mobility assay utilizing probes/primers from the core/E1 region of the HCV 15 genome. The documented antigenic differences between HCV genotypes would have usefulness not only in blood donor screening and in predicting response to IFN treatment, but also for the designated composition of candidate vaccines for HCV in different countries, choice of therapeutic vaccines, as well as in the identificaiton of new 20 genotypes. Other methods have been proposed to identify the main genotypes infecting populations, based on analysis of different regions of the genome, such as RFLP. See Davidson et al., *J. Gen Virol.* (1995) 76:1197-1204 for discussion of genotyping HCV using RFCP of sequences camplified form the 5' non-coding region (NCR).

The known nucleic acid based methods of genotyping require a sub-type specific 25 RT-PCR(reverse transcriptase-PCR) primers (see Okamoto (1992) *J. Gen Virol* 73:673-679) U.S. Patent 5,427,909; (2) specific probes (G. Marteen, et al., Line probe assay); (3) restriction site polymorphism (a function of the nucleotide sequence (nt)) or (4) direct sequences to determine genotype. The analysis of the 5' NC sequence with RFLP is easy to perform, but does not accurately predict all HCV genotypes, and, some subtypes may be misclassified. For example, the change in sequence between 1a and 1b recognized by 30 the restriction enzyme is not absolute and sequences other than 1a and 1b, and 2a and 2b

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are misclassified. For example, type 1c would appear as type 1a, type 2c as either type 2a or 2b. See Cammarota et al., *J. Clin. Microb.*, (1995) 33:2781-2784. For this reason, RFLP is not able to detect "escape" species, new divergent species, or epidemiological trends. It is likely that a typing method like RFLP will have to be continuously modified 5 to accommodate the rapidly increasing information collected on HCV sequence heterogeneity.

As above-mentioned, when using the nucleic acid based methods of genotyping, one obtains a result of either a type or subtype or a negative that is "untypeable" result. See, e.g. Cammarota, et al., *J. Clin. Microb.* (1995) 33:2781-2784, isolates that remained 10 untyped by genotype-specific PCR were classified subtype 2c on the basis of sequence analysis of PCR amplicons obtained from the core and NS5 genes. This problem is avoided by using the presently claimed invention to determine HCV genotypes by choosing RT-PCR primers in the C-terminus or core/mid 2/3 of E1. In addition, the subtype of the isolate can be accurately determined using the present invention of HCV 15 genotyping and isolates can be detected, even those less than in approximately 30% divergent, enabling the characterization of new sub-types without sequencing.

Heteroduplex Tracking or Mobility Assay

The method of determining the genotype of HCV in the present invention utilizes 20 minor variants in complex quasispecies. One such technique is the heteroduplex tracking assay (HTA). HTA, well known in the art for use with HIV, (see e.g., Delwart, et al., *J. Virol.* (1994) 68:6672-6683; Delwart, et al., *Science* (1993) 262:1257-1261; Delwart, et al., *PCR Methods and Applications* 4:S202-S216 (1995) Cold Springs Harbor; and Delwart, et al., *Heteroduplex Mobility Analysis HIV-1 env Subtyping Kit Protocol* 25 Version 3, each of which is incorporated herein by reference in its entirety), grew out of the observation that when sequences were amplified by nested PCR from peripheral blood mononuclear cells of infected individuals, related DNA products coamplified from divergent templates could randomly reanneal to form heteroduplexes that migrate with reduced mobility in neutral polyacrylamide gels. Using these techniques, one can 30 establish genetic relationships between multiple viral DNA template molecules.

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HTA in particular utilizes a first PCR product as a labeled probe, it may be radioactive, which is mixed with an excess (driver) of an unlabeled PCR product from a different source, i.e., the source for which typing is desired. The probe sequences are then driven completely into heteroduplexes with the driver, and are separated on the basis 5 of size. An autoradiogram for example of the resulting polyacrylamide gel reveals only these heteroduplexes and provides a visual display of the relationship between the two virus populations under study. The fact that heteroduplexes migrate with distinct mobilities indicates that the strand-specific composition of mismatched and unpaired nucleotides affects their mobility.

10 An exponential equation described in Delwart et al., is then used to describe a curve fitting the experimental data from pairwise analysis of genes of known sequence. In the present invention, the equation is used to estimate the genetic distance between the known genotypes of the probes and the unknown genotypes of the patient samples.

15 Primers for Use in the HTA

It was determined that the E1 or core region could be the best region in to study the HCV heterogeneity, thus the E1 region became the choice for primers in the present invention. The use of the partial E1 sequence, the most heterogeneous region of the genome for the present invention, as well as a longer fragment, i.e. 400nt, although it 20 could have been as long as 1000nt, enabled the design of probes which do not cross hybridize among sub-types/types and thus allow accurate geneotyping. By flanking the heterogeneous region, conserved nt sequences for sense and antisense primers were identified. Preferably, a combination of universal sense and type specific antisense primers for the first PCR round and a universal antisense and type specific sense primers 25 for the second round were utilized. The PCR need not be two rounds and the primers are not limited to the above-described combination. The preferred combination, however, enabled the preparation of single stranded probes and minimized the number of PCR primer combinations.

Preferred probes are sequences in the core and E1 regions of which the 30 sequences for a wide range of genotypes are published and grouped into at least 12

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distinct genotypes and subtype: I/1a, II/1b, III/2a, IV/2b, 2c, 3a, 4a, 4b, 4c, 4d, 5a, 6a.

The nucleotide sequence identities of the E1 gene among HCV isolates of the same genotype ranges from 88.0% to 99.1%, whereas those of HCV isolates of different genotypes ranges from 53.5 to 78.6%. The degree of variation for good discrimination of

5 heteroduplex in neutral polyacrylamide gels is comfortably within the range of 3-20%, so that is likely that divergent templates reanneal to form a heteroduplex if they are of the same subtype. For this reason, a single stranded 32p labelled DNA probe was used so that if the formation of the heteroduplex is impossible, the ss-DNA probe could likely not reanneal and form a homoduplex band. Without direct sequencing, the present

10 invention can rapidly give not only a certain identification of the subtypes, but also the genetic relations inside the same subtypes. For example, the genotypes analyzed, i.e., (1a, 1b, 2a, 2b, 3a) showed no overlapping between different subtypes.

Further since isolates approximately 30% divergent can be visualized on the gel-
new subtypes can be visualized and the distribution of isolates in a population could be
15 characterized and populations or individual isolates can be followed in population or in individuals in epidemiological studies.

HCV Genotyping Kits

A kit for determining the genotype of HCV is within the scope of this invention.

20 As described for HIV in Delwart et al, Heteroduplex Mobility Analysis HIV-1 env Subtyping Kit Protocol Version 3, such a kit would include the specific primers. Preferred primers are from the core and E1 region of the HCV genome. If two stages of PCR are desired, the first round primers could include for example a universal sense probe, preferably located in the core/E1 region of the HCV genome. One such universal
25 primer is located from nucleotide 508 to 529 of HCV-1 and is shown in Table 1.

Coupled with the universal primer could be a type specific antisense primer also. preferably located in the core/E1 region of the HCV genome. Examples of these primers are from nucleotides 1032 to 1012 for type 1, type 2a, type 2b and type 3a of the HCV genomes and are also shown in Table 1.

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If a second round of PCR is desired, the second round primers would likewise be from the core/E1 region of the HCV genome. Preferred second round primers could include a universal antisense primer from nucleotides 978 to 958 of the HCV-1 genome, this primer is shown in Table 1. In addition the second round primers could include a type specific sense primer from the core/E1 region. Preferred second round type specific sense primers are from nucleotides 536 to 557 of HCV genomes type 1, type 2 or type 3, and are shown in Table 1.

5 The first or second round of primers may be sufficient to amplify the viral RNA without using a second round of PCR if the concentration of the virus is sufficiently high, 10 ie., nested PCR is not necessarily required, what is required is PCR products in 100x excess of probe.

15 An HCV genotyping kit of the present invention would also include subtype references which may change as new subtypes are discovered and evaluated for use in the kit. Use of more than one reference from a given subtype is recommended because comparison to a single reference does not always provide an unambiguous result.

The foregoing discussion and following examples only illustrate the invention, persons of ordinary skill in the art will appreciate that the invention can be implemented in other ways, and the invention is defined solely by reference to the claims.

20

Example 1

Patient samples

35 hemodialyzed patients undergoing regular hemodialysis were studied: 20 men (57%) and 15 women (43%) with a mean age of 64.8 ± 13 years. Serum samples were collected in August 1995, divided into aliquots and stored at -80 degrees Celsius. 26 patients were anti-HCV ELISA positive and 9 anti-HCV ELISA negative. 25 of the 26 ELISA positive were also RIBA III positive, while 1 was indeterminate. The 9 ELISA negative were all RIBA III negative. 15 patients were HCV-RNA 5' UTR and E1 PCR positive. By direct sequencing of 15 5' NCR products, 5 patients resulted type 1; 3 patients type 2; and 7 patients type 3.

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Example 2

cDNA and PCR

HCV-RNA was extracted at least two different times using a Stratagene reagent from a Strategene RNA Isolation Kit (Chomezynsky and Sacchi method).

5 RNA extracted from 20ul of plasma that was reverse transcribed in a 25 ul of cDNA mixture (BRL cDNA synthesis kit, 8085SB) using 100 pmol of PCR primers. The cDNA mixture was boiled for 5 minutes, quick-cooled on ice and added to the PCR cDNA reagents with final concentrations according to the Perkin PCR kit (N801-0055) specification. 40 PCR cycles (94 degrees Celsius for 10 seconds, 55 degrees Celsius for 10 30 seconds and 72 degrees Celsius for 30 seconds were performed. Ten ul of the first PCR reaction mixture was added to a second PCR reaction mixture containing nested PCR primers and was amplified for 40 cycles as indicated above.

15 The first extraction was used for the nested-PCR reaction with primers specific for the 5' NCR as previously described in Shimizu et al, PNAS (1992) 5477-5481 and this product was directly sequenced and used for the RFLP. RNA from the same extraction was used for HTA using core/E1 primers. A second RNA extraction was performed for RFLP and/or HTA to confirm the results. The primers used for the HTA are listed in Table 1. The nested pairs of PCR primers used to obtain these E1 products were different for the types 1, 2a, 2b, and 3a. The universal sense probe for the first 20 round of amplification corresponds to 5'-3' nt 508-529, amino acids 170-176, of Choo, et al., PNAS, 1991, while the universal antisense primer for the second round of amplification corresponds to nt 978-958, amino acids 320-326 of Choo, et al., PNAS, 1991.

25 When the ssDNA DNA probes were prepared for use in the HTA, one of the primers for the nested PCR was biotinylated. See e.g. SEQ ID NO:6 in Table 1.

15

Table 1

	HCV-1	5' → 3' nt	3' → 5' nt	~ Amino Acid	Primer Type
5	(SEQ ID NO:1) Purified, C170S	508-529	529-508	170-176	Universal sense probe PCR I
10	(SEQ ID NO:2) Purified, E338A1	1032-1012	1012-1032	338-344	Type 1 antisense PCR I
	(SEQ ID NO:3) Purified, E338A2a	1032-1012	1012-1032	338-344	Type 2a antisense PCR I
15	(SEQ ID NO:4) Purified, E338A2b	1032-1012	1012-1032	338-344	Type 2b antisense PCR I
	(SEQ ID NO:5) Purified, E338A3a	1032-1012	1012-1032	338-344	Type 3a antisense PCR I
20	(SEQ ID NO:6) Purified, E320A	978-958	958-978	320-326	Universal antisense PCR II
25	(SEQ ID NO:7) Purified, C179S1	536-557	958-978	179-186	Type 1 sense PCR II
	(SEQ ID NO:8) Purified, C179S2	536-557	958-978	179-186	Type 2 sense PCR II
30	(SEQ ID NO:9) Purified, C179S3	536-557	958-978	179-186	Type 3 sense PCR II

Example 3

35

HTA

The single stranded probes were prepared by RT-PCR of HCV ELISA and RIBA positive sera of known genotypes with the same PCR primers described, as above, except that one of the primers 320A was biotinylated. ssDNA probes were generated with the Dynabeads M-280 Streptavidin following the protocol of Heng Pan and Eric Delwart.

40 The non-biotinyl single strand was eluted from the magnetic bead/streptavidin column. Probes were generated from 20 ng of ssDNA of the different genotypes and end labeled using T4 polynucleotide kinase (Gibco BRL) and 100 microCi of 32P ATP and then column purified. The kinase probe was separated from 32P ATP using a Pharmacia Bio Sepharose column. The 32P-labeled single strand probes were mixed with a 100-fold excess driver, and the PCR products were generated from the patient samples or the control serum/plasma. Hybridization was in 2 x SSC. The mixtures were put on a 94 degree Celsius heat block for 3 minutes. They were then transferred to a 55 degree

16

Celsius heat block for at least 2 hours. The entire reaction volume was loaded on 1mm thick, 6% polyacrylamide MDE gel (Baker) and electrophoresed for 16 h at 500V. The gel was vacuum dried at 80 degrees Celsius on filter paper and exposed to X-ray film. The genotypes of each of the samples were determined based on the Delwart method.

5 Table 2 depicts the genotype results determined by using HTA.

Figures 1A-1E are autoradiograms showing each of the single strand probes in Table 1, that is the probes specific known for genotypes 1a, 1b, 2a, 2b, 3a in Figures 1A-E respectively, see the lane on the far left of the MDE gel. The homoduplex(h) (ss probe to the double stranded RT-PCR product form which it was derived) is shown adjacent to 10 the probe. RT-PCR products from the 15 dialysis patients (nos. 1, 2, 3, 4, 7, 18, 20, 22, 23, 24, 26, 28, 30, 33, 35) hybridized to the probe is designated also as the appropriate 15 lane in each Figure.

As can be seen in Figures 1A-1E, Type 1 ss subtypes probes were specific for 20 each type 1 sub-type and did not cross hybridize with other subtypes 1b, 2a, 2b, 3a (2a, 2b not shown). Type 3a ss sub-type specific probe was also specific for subtype 3a and did not cross hybridized with 1a, 2c, or 2a, 2s isolates (data not shown). ss Sub-type 2 probes do not cross hybridize with each other (data not shown) but did cross-hybridized 25 with subtype 2c isolates; however, the distance between the homoduplex and the 2c isolates indicates a high degree of divergence suggesting that patients 23, 30 and 33 had different sub-types. The virus in sera 23, 30 and 33 was confirmed by sequencing the partial E1 to be most closely related to sub-type 2c (see figure 2b) but was ambiguous by 30 51UTR sequencing, See Figure 2D.

Isolates 23, 30 and 33 hybridized with the 2a probe, while only 30 and 33 hybridized to the 2b probe. The gels also indicate that isolate 30 is more closely related 25 to 2a than to 2b. Therefore, while all three sera are clearly type 2 non-a, non-b subtype, they are not all equally divergent from types 2a and 2b. As seen in Figures 1B and 1D, patient 4 appears to be co-infected with types 1b and a non-a, non-b type subtype.

The 1b probe was derived from a patient (JK 16) and appeared to have two viral 30 genomes which is reflected in the homoduplex lane (h) and therefore each 1b patient has two bands.

The ss probe 3a was derived from a plasmid clone of one RT-PLR product from a type 3a individual (JK3a), see Fig. 1E, lane h, therefore, multiple bands in lane 22 most likely reflect two closely related viruses in this patient.

It appeared that most often patients had unique viral isolates. It is possible that 5 patients 3 and 18 had identical or highly related virus isolates. Similarly, patients 20 and 26 had the same type 3a viral isolate and patients 2 and 4 has the same type 1b isolate based on the co-migration of the bands on MDE gels.

Figures 2a-2c depict phylogenetic trees, dendograms, showing the genetic relatedness of each of the partial E1 nucleotide sequences. These denrograms were 10 constructed by pairwise progressive alignment of the nucleotide sequences to one another by using the computer software program GeneWorks Unweighted Pair Group Methods with Arithmetic mean, as described in Weiner, et al., *J. Virol.* 67: pg. 4365-4368 (1993). The dendograms, in Figures 2a-2c were formed by comparing partial E1 sequences of putative type 1 (nt 625-93), type 2 (nt 583-915) or type 3 (nt 558-834) isolates from the 15 dialysis patients, as determined by sequence analysis to published genotype sequences for type 1a (HCV-1) (Choo, et al. PNAS 1991); 1b (HCV-J) (Kato et al.); 2a (HC-J6)(Okamoto, et al (1992); 2b (HC-J8)(Okamoto, et al, 1992); 2c (Bukh, et al.PNAS 1993) and 3a (NZL-1) (Sakamoto, et al. 1994) over the same region of the genome.

Figure 2D is a dendrogram formed as above-described by comparing either 20 partial 5' UTR sequences of isolates 23, 30 and 33 with published type 1, 2 and 3 (nt-274 to -81) genotype sequences for the same region of the genome.

The results of the RFLP and HTA were compared and are presented in Table 2.

Table 2

Comparison of Partial E1 HTA and RFLP Genotyping Results

5	Patient	HTA	RFLP
10	1	1b	1b
	2	1b	1b
	3	3a	3a
	4	1b	1b
	7	3a	3a
	18	3a	3a
15	20	3a	3a
	22	3a	3a
	23	2?*	2a
	24	1b	1b
	26	3a	3a
	28	1b	1b
20	30	2?*	2a
	33	2?*	2a
	35	3a	3a
* sample is neither 2a nor 2b			

25

The partial E1 sequences depicted in Figures 3a-3d confirm the HTA sub-type designations given in Table 2 and definitively show that patients 23, 30 and 33 are most closely related to 2c with 33 being the most distantly related to 2c. (18.6% divergent).

30

The RFLP results using ScrFI (see Davidson, et al., J. Gen. Virol. (1995) 76:1197-1204) wrongly designated 23, 30 and 33 as type 2a. This wrong designation is reflected in Figure 2D which shows that based on the 5' UTR nt sequence, the computer did not accurately sub-type HCV 2c due to insufficient nt divergence in this region of the genome.

35

The present invention of HTA utilizing primers for the core and envelope region allowed for 3 levels of characterization of HCV genomes. The first was type specificity in the choice of RT-PCR primers. The second was sub-type specificity, based on choosing primers in the core/E1 region, and from a region greater than 400 nt, which

19

resulted in a lack of cross-hybridization between sub-type probes, e.g. 1 and 3, 2a, 2b; and a high degree of heterogeneity to maximize differences between genotypes (lack of cross-hybridization). Finally, isolate specificity was determined by the distance from the homoduplex as exemplified in Figures 1.E - 1-E. Other genotyping methods do not have 5 the ability to analyze isolate differences

20
SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: CHIRON CORPORATION
- (ii) TITLE OF INVENTION: HETERODUPLEX TRACKING ASSAY (HTA) FOR GENOTYPING HCV
- (iii) NUMBER OF SEQUENCES: 52
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Chiron Corporation
 - (B) STREET: 4560 Horton Street - R440
 - (C) CITY: Emeryville
 - (D) STATE: California
 - (E) COUNTRY: USA
 - (F) ZIP: 94608-2916
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: Unassigned
 - (B) FILING DATE: Even date herewith
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Harbin, Alisa A.
 - (B) REGISTRATION NUMBER: 33,895
 - (C) REFERENCE/DOCKET NUMBER: 1226.100
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (510) 923-3274
 - (B) TELEFAX: (510) 655-3542
 - (C) TELEX: N/A

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTGGTTGCT CTTTCTCTAT CT

22

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs

21

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GATGGCTTGT GGGATCCGGA G

21

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GATGACCTCG GGGACGCGCA T

21

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GACCAGTTCT GGAACACGAG C

21

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAAGGTCTGG GGTAAACGCA G

21

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

22

(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCAGTTCATC ATCATATCCC A

21

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGCCCTGCT CTCTTGCTTG AC

22

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTGCTCTTCT GTCGTGGTC AC

22

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTGCTCTGTT CTCTTGCTTA AT

22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 306 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

23

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACTCAAGCA TTGTGTATGA AGCGGCGGAC ATGATCATGC ACACCCCCGG GTGCGTGCCA	60
TGCGTCCGGG AGGGCAATCT CTCCCGCTGC TGGGTAGCGC TCACCTCCAC GCTCGCGGCC	120
AGAAACAGCA GCGTCCCTAC TACGACAATA CGACGCCATG TCGACTTGCT AGTAGGAGCG	180
GCTGCTTTTT GCTCCGCCAT GTACGTGGGG GACCTCTGCG GATCTATTT CCTCGTCTCC	240
CAAATGTTCA CCTTCTCGCC CCGCCGGCAT CATACTGAC AGGACTGCAA TTGCTCGATC	300
TATCCC	306

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AACTCAAGCA TCGTGTATGA GGCAGCGGAA GTGATCATGC ACATTCCCGG GTGCGTGCCC	60
TGCGTTCGGG AGAGCAATCT CTCCCGCTGC TGGGTAGCGC TCACCCCCAC ACTCGCGGCC	120
AGGAACAGCA GCGTCCCCAC CACGACAATA CGACGCCACG TCGACTTGCT CGTTGGGGCG	180
GCTGCCTTCT GCTCCGCTAT GTATGTGGGG GATCTCTGCG GATCTGTTT CCTTGTCTCC	240
CAAATGTTCA CCTTTTCGCC TCGCCGGCAT GAGACAGTAC AGGACTGCAA TTGTTCAATC	300
TATCCC	306

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AACTCAAGCA TAGTATATGA GGCAGCGGAC ATAATCATGC ATACCCCCGG GTGCGTGCCC	60
TGTGTTCCGGG AGGTCAACTC CTCCCGCTGC TGGGCAGCGC TCACCCCTAC GCTCGCGGCC	120
AGGAACCTCCA GCGTCCCCAC TACGACAATA CGACGCCACG TCGACTTGCT CGTTGGGGCG	180

GCTGCTTCT	GCTCCGCTAT	GTACGTGGGG	GATCTATGCG	GATCTGTTCT	ACATGTCTCT	240
CAGCTGTTCA	CCTTCTCACCC	TCGCCGGCAC	GAGACAGTGC	AGGACTGCAA	TTGTTCAATC	300
TATCCC						306

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AACACGAGCA	TTGTGTATGA	GGCAGCGGAC	TTGATCATGC	ACGTCCCCGG	GTGCGTGC	60
TGCGTTCGGG	AGGGCAACTC	CTCCCGATGC	TGGGTAGCGC	TCACTCCCAC	GATCGCGGCC	120
AGGAACAGCA	GTGTCCCCGT	TACGACCATA	CGACGCCACG	TCGATTTGCT	CGTTGGGGCG	180
GCTGCTCTTT	GCTCCGCCAT	GTACGTGGGG	GATCTCTGCG	GATCTGTCTT	CCTCGCTTCC	240
CAGTTGTTCA	CTTTCTCGCC	TCGCCAGCAT	CAGACGGTAC	AGGACTGCAA	CTGCTCAATC	300
TATCCC						306

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AACTCAAGCA	TCGTGTATGA	GGCGGCGGAA	GTGATCATGC	ACATTCCTGG	GTGCGTGC	60
TGCGTTCGGG	AGGGCGACTT	CTCCCGCTGC	TGGGTAGCGC	TCACCCCCAC	ACTCGCGGCC	120
AGGAATAACA	GCGTCCCCAC	TACGACAATA	CGACGCCACG	TCGACTTGCT	CGTTGGGGCG	180
GCTGCCTTCT	GCTCCGCTAT	GTACGTGGGG	GATCTCTGCG	GATCTGTTTT	CCTTGTCTCC	240
CAACTGTTCA	CCTTTTCGCC	TCGCCGGCAT	GCGACAGTAC	AGGACTGCAA	TTGTTCAATC	300
TATCCC						306

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 306 base pairs

25

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(iii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AACTCGAGTA TTGTGTACGA GGCGGCCGAT GCCATCCTGC ACACCTCCGGG GTGCGTCCCT	60
TGCGTTCGTG AGGGCAACGC CTCGAGGTGT TGGGTGGCGA TGACCCCTAC GGTGGCCACC	120
AGGGATGGCA AACTCCCCGC GACGCAGCTT CGACGTCACA TCGATCTGCT TGTCGGGAGC	180
GCCACCCCTCT GTTCGGCCCT CTACGTGGGG GACCTATGCG GGTCTGTCTT TCTTGTGGC	240
CAACTGTTCA CCTTCTCTCC CAGGCGCCAC TGGACGACGC AAGGTTGCAA TTGCTCTATC	300
TATCCC	306

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 306 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AACTCAAGTA TTGTGTATGA GGCAGCGGAC ATGATCATGC ACACCCCCGG GTGCGTGCCT	60
TGCGTCCGGG AGAGTAATTT CTCCCGTTGC TGGGTAGCGC TCACTCCCAC GCTCGCGGCC	120
AGGAACAGCA GCATCCCCAC CACGACAATA CGACGCCACG TCGATTGCT CGTTGGGGCG	180
GCTGCTCTCT GTTCCGCTAT GTACGTTGGG GATCTCTGCG GATCCGTTTT TCTCGTCTCC	240
CAGCTGTTCA CCTTCTCACC TCGCCGGTAT GAGACGGTAC AAGATTGCAA TTGCTCAATC	300
TATCCC	306

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 306 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AATGATAGCA TTACCTGGCA ACTCCAGGCT GCTGTCCTCC ACACCCCCGG GTGCGTCCCG	60
---	----

TGCGAGAAAAG TGGGAATAAC ATCTCGGTGC TGGATACCGG TCTCACCGAA TGTGGCCGTG	120
CAGCAGCCCG GCGCCCTCAC GCAGGGCTTA CGGACGCACA TTGACATGGT TGTGATGTCC	180
GCCACGCTCT GCTCCGCTCT TTACGTGGGG GACCTCTGCG GTGGGGTGAT GCTTGCAGCC	240
CAGATGTTCA TTGTCTCGCC ACAGCACCAC TGGTTTGTC AAGACTGCAA TTGCTCCATC	300
TACCCCT	306

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 306 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AACAAACAGCA TCACCTGGCA GCTCACTGAC GCAGTTCTCC ATCTTCTGG ATGCGTCCCA	60
TGTGAGAATG ATAATGGCAC CTTGCATTGC TGGATACAAG TAACACCCAA CGTGGCTGTG	120
AAACACCGCG GTGCGCTCAC TCGTAGCCTG CGAACACACG TCGACATGAT CGTAATGGCA	180
GCTACGGCCT GCTCGGCCTT GTATGTGGGA GATGTGTGCG GGGCCGTGAT GATTCTATCG	240
CAGGCTTCA TGGTATCACC ACAACGCCAC AACTTCACCC AAGAGTGCAA CTGTTCCATC	300
TACCAA	306

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 306 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATAGCAGTA TTGTGTATGA GGCGATGAT GTCATTCTGC ACACACCCGG CTGTGTACCT	60
TGTGTCAGG ACGGCAATAAC ATCTACGTGC TGGACCCAG AGACACCTAC AGTGGCAGTC	120
AGGTACGTG GAGCAACTAC TGCTTCGATA CGCAGTCATG TGGACCTATT AGTAGGCGCG	180
GCCACGATGT GCTCTGCGCT CTACGTGGGT GATATGTGTG GGGCTGTCTT TCTCGTGGGA	240
CAAGCCTTCA CGTCAGACC TCGACGCCAT CAAACGGTCC AGACCTGTAA CTGCTCGCTG	300
TACCCA	306

(2) INFORMATION FOR SEQ ID NO:20:

27

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 333 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CGCAACTCCA CGGGGCTTTA CCACGTCACC AATGATTGCC CTAACCTGAG TATTGTGTAC	60
GAGACGGCCG ATGCCATCCT GCACACTCCG GGGTGCCTCC CTTGTGTTCG CGAGGGCAAC	120
GCCTCGAGGT GTTGGGTGGC GATGACCCCT ACGGTGGCCA CCAGGGATGG CAAACTCCCC	180
GCGACGCAGC TTGACGTCA CATCGATCTG CTTGTCGGGA GCGCCACCCCT CTGTTGGCC	240
CTCTACGTGG GGGATCTGTG CGGGTCTGTC TTTCTTGTG GCAAATGTT TACCTTCTCT	300
CCCAGGCGCC ACTGGACGAC GCAAGGTTGC AAT	333

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 333 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AAGAACACCA GCGACAGCTA CATGGTGACC AATGACTGCC AAAATGACAG CATCACCTGG	60
CAGCTTGAGG CTGCGGTCCCT CCACGTCCCC GGGTGCCTCC CGTGCAGAG AGTGGAAAT	120
ACATCTCGGT GCTGGATACC GGTCTCACCA AACGTGGCTG TGCGGCAGCC CGGCGCCCTC	180
ACGCAGGGCT TGCGGACGCA CATCGACATG ATTGTGATGT CCGCCACGCT CTGCTCCGCT	240
CTCTACGTGG GGGACCTCTG TGGCGGGATG ATGCTCGCAG CCCAGATGTT CATCGTTCTG	300
CCGCAGAACCC ACTGGTTCGT GCAGGAATGC AAT	333

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 333 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

28

AGGAACATCA GTTCTAGCTA CTACGCCACT AATGACTGCT CGAACAAACAG CATCACCTGG	60
CAGCTCACCA ACCCAGTTCT CCACCTTCCC GGATGCGTCC CATGTGAGAA TAATAATGGC	120
ACCTTGCATT GCTGGATACA AGTAACACCT AATGTGGCCG TAAAACATCG CGGCGCACTC	180
ACTCACAACC TGGCGACACA TGTCGACATG ATCGTAATGG CAGCTACGGT CTGTTCGGCC	240
TTGTACGTAG GAGACGTGTG TGGGGCTGTG ATGATTGTGT CTCAGGCCCT TATAATATCA	300
CCAGAACACC ATAACATCAC CCAAGAGTGC AAC	333

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AAGGACACCG GCGACTCCTA CATGCCGACC AACGATTGCT CCAACTCTAG TATCGTTGG	60
CAGCTTGAAG GAGCAGTGCT TCATACTCCT GGATGCGTCC CTTGTGAGCG TACCGCCAAC	120
GTCTCTCGAT GTTGGGTGCC GGTTGCCCGG AATCTGCCA TAAGTCAACC TGGCGCTCTC	180
ACTAAGGGCC TCGGAGCACA CATCGATATC ATCGTGTATGT CTGCTACGGT CTGTTCTGCC	240
CTTTATGTGG GGGACGTGTG TGGCGCGCTG ATGCTGGCCG CTCAGGTCGT CGTCGTGTG	300
CCACAAACACC ATACGTTGTG CCAGGAATGC AAC	333

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

AAAAAACACCA GCATCTCCTA TATGGCGACC AACGACTGCT CCAATTCCAG CATCGCTTGG	60
CAGTTTGACG GCGCAGTGCT CCATACTCCT GGATGTGTCC CTTGCGAACG GACCGGCAAC	120
GCGTCCCGGT GTTGGGTGCC GGTTGCCCGG AATGTGGCTA TAAGACAACC CGGCGCCCTC	180
ACTAAGGGCA TACGAACGCA CATTGATGTC ATCGTAATGT CTGCTACGCT CTGTTCTGCC	240
CTTTACGTGG GGGACGTGTG TGGTGCCTG ATGATTGCCG CTCAGGTCGT CATTGTGTCT	300

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CCGCAGCATC ACCACTTTGT CCAGGACTGC AAT

333

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AAGAACACCA GCGACTCCTA CATGGCGACT AACGACTGCT CTAACCTCCAG CATCGTTGG	60
CAGCTTGAGG ACCCAGTGCT CCATGTCCT GGATGTGTCC CTTGTGAGAA GACTGGCAAT	120
ACGTCTCGGT GCTGGGTGCC GGTTACCCCC AATGTGGCTA CAAGTCAACC CGGCGCTCTC	180
ACCAGGGGCT TGGCGACGCA CATCGATGTC ATCGTGTATGT CAGCCACGCT CTGCTCCGCT	240
CTCTATGTGG GGGACGTGTG TGGCGCGTTG ACGATAGCCG CTCAGGTTGT CATCGTATCG	300
CCACGGCACC ACCACTTTGT CCAGGACTGC AAT	333

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAGAACACCA GCACCTCCTA CATGGTACT AACGATTGCT CCAACTCCAG CATCGTTGG	60
CAAACCTGAAG GCGCAGTGCT CCATGTCCT GGATGTGTCC CTTGTGAGCA GATCGGCAAC	120
GTGTCTCAGT GTTGGGTGCC GGTTACCCCC AATATGGCCA TAAGTACACC CGGCGCTCTC	180
ACTAAGGGCT TGGCAACGCA CATCGACGGC ATCGTGTATGT CCGCTACGCT CTGTTCTGCC	240
CTTTATGTGG GGGACGTGTG TGGCGCGTTG ATGATAGCCG CCCAGGTCGT CATCGTATCG	300
CCACAGCACC ACCACTTTGT CCACGACTGC AAC	333

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CGCAACTCCA CGGGGTTTA CCACGTCACC AATGATTGCC CTAACTCGAG TATTGTGTAC	60
GAGGCGGCCG ATGCCATCCT GCACACTCCG GGGTGCCTCC CTTGCCTTCG TGAGGGCAAC	120
GCCTCGAGGT GTTGGGTGGC GATGACCCCT ACGGTGCCA CCAGGGATGG CAAACTCCCC	180
GCGACGGCAGC TTGACGTCA CATCGATCTG CTTGTCGGGA GCGCCACCCCT CTGTCGGCC	240
CTCTACGTGG GGGACCTATG CGGGTCTGTC TTTCTGTGCG GCCAACTGTT CACCTTCTCT	300
CCCAGGGGCC ACTGGACGAC GCAAGGTGTC AAT	333

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CGAACGTGT CCGGGATATA CCATGTCACG AACGACTGCT CCAAACCAAG TATTGTGTAT	60
GAGGCAGCGG ACATGATCAT GCACACCCCC GGGTGCCTGC CCTGCCTCCG GGAGAGTAAT	120
TTCTCCCGTT GCTGGGTAGC GCTCACTCCC ACGCTCGCGG CCAGGAACAG CAGCATCCCC	180
ACCACGACAA TACGACGCCA CGTCGATTTG CTCGTTGGGG CGGCTGCTCT CTGTCGGCT	240
ATGTACGTG GGGATCTCTG CGGATCCGTT TTTCTCGTCT CCCAGCTGTT CACCTTCTCA	300
CCTCGCCGGT ATGAGACGGT ACAAGATTGC AAT	333

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

-- --

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AGGAACATTA GTTCTAGCTA CTACGCCACT AATGATTGCT CAAACAAACAG CATCACCTGG	60
CAGCTCACTG ACGCAGTTCT CCATCTTCCT GGATGCCTCC CATGTGAGAA TGATAATGGC	120
ACCTTGCATT GCTGGATACA AGTAACACCC AACGTGGCTG TGAAACACCG CGGTGCGCTC	180
ACTCGTAGCC TGCACACACA CGTCGACATG ATCGTAATGG CAGCTACGGC CTGCTCGGCC	240

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TTGTATGTGG GAGATGTGTG CGGGGCCGTG ATGATTCTAT CGCAGGCTTT CATGGTATCA	300
CCACAACGCC ACAACTTCAC CCAAGAGTGC AAC	333

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGGAATACGT CTGGCCTCTA CGTCCTTACC AACGACTGTT CCAATAGCAG TATTGTGTAT	60
GAGGCCGATG ATGTCATTCT GCACACACCC GGCTGTGTAC CTTGTGTCCA GGACGGCAAT	120
ACATCTACGT GCTGGACACCC AGTGACACCT ACAGTGGCAG TCAGGTACGT CGGAGCAACT	180
ACTGCTTCGA TACCGCAGTCA TGTGGACCTA TTAGTAGGCG CGGCCACGAT GTGCTCTGCG	240
CTCTACGTGG GTGATATGTG TGGGGCTGTC TTTCTCGTGG GACAAGCCTT CACGTTCAGA	300
CCTCGACGCC ATCAAACGGT CCAGACCTGT AAC	333

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

AAGAACATCA GTACCGGCTA CATGGTGACC AACGACTGCA CCAATGATAG CATTACCTGG	60
CAAATCCAGG CTGCTGTCCT CCACGTCCCC GGGTGGCTCC CGTGCAGAAA AGTGGGGAAAT	120
ACATCTCGGT GCTGGATACC GGTCTCACCG AATGTGGCCG TGCAGCAGCC CGGCGCCCTC	180
ACGCAGGGCT TACGGACGCA CATTGACATG GTTGTGATGT CCGCCACGCT CTGCTCCGCT	240
CTTTACGTGG GGGACCTCTG CGGTGGGTG ATGCTTGCAG CCCAGATGTT CATTGTCTCG	300
CCACAGCACC ACTGGTTTGT GCAAGACTGC AAT	333

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACATTATTG TGTATGAGGC CGATGACGTC ATCCTGCACA CGCCCGGCTG	120
TGTACCTTGT GTTCAGGACG GAAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGTGGAG CAACCACCGC TTCAATACGC AGCCACGTGG ACCTATTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACATCATTG TGTATGAGGC CGATGACGTC ATCCTGCACG CACCCGGCTG	120
TGTACCTTGT GTTCAGGACG GAAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGTGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTATTAGT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AATATTATTG TGTATGAGGC CGACGACGTC ATCCTGCACG CCCCCGGCTG	120
TGTACCTTGT GTTCAGGACG GAAATACATC CACGTGCTGG ATCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGCCGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTGTTAGT	240

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GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT

277

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACATTATTG TGTATGAGGC CGATGACGTC ATCCTGCACA CACCCGGCTG	120
TGTACCTTGT GTTCAGGACG GCAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT	180
ATCAGTCAGG TACGTGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTACTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCCAAT AACAGTATTG TGTATGAGGC CGATCACGTC ATCCTGCACA CACCCGGCTG	120
TGTACCTTGT GTTCAAGCCA ACAATAAAC CAAATGCTGG ACCCCAGTGA CACCTACAGT	180
ATCAGTCAGG TACGTGGAG CAACCACCGC TTCAATACGC AGCCATGTGG ACCTACTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

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TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TTACCAACGA	60
CTGTTCTAAT AACATTATTG TGTATGAGGC CGATGACGTC ATCCTGCACA CACCCGGCTG	120
TGTACCTTGT GTTCAGGACG GCAATGCATC CACGTGCTGG ACCCCAGTAA CACCTACAGT	180
ATCAGTCAGG TACGTGGAG CAACCACCGC TTCAGTACGC AGCCATGTGG ACCTACTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA TGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TCATCCAACA TCTAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTATGTCC TCACCAACGA	60
CTGTTCAAC AACATTATTG TGTATGAGGC CGATGACGTC ATTCTGCACA CGCCCGGCTG	120
CGTACCTTGT GTACAGGACG GCAATACATC CACGTGCTGG ACCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGTGGAG CAACTACCGC TTCAATACGC AGCCATGTGG ACCTATTATT	240
GGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TCATCCAGCA GCCAGTCTAG AGTGGCGGAA TACGTCTGGC CTCTACGTCC TTACCAACGA	60
CTGTTCAAT AGCAGTATTG TGTATGAGGC CGATGATGTC ATTCTGCACA CACCCGGCTG	120
TGTACCTTGT GTCCAGGACG GCAATACATC TACGTGCTGG ACCCCAGTGA CACCTACAGT	180
GGCAGTCAGG TACGTGGAG CAACTACTGC TTCGATACGC AGTCATGTGG ACCTATTAGT	240
AGGCGCGGCC ACGATGTGCT CTGCGCTCTA CGTGGGT	277

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid

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(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(i-i) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

TGTGCCCGCT TCGGCCTACC AAGTGCAGCA CTCCACGGGG CTTTACCAACG TCACCAATGA	60
TTGCCCTAAC TCGAGTATTG TGTACGAGGC GGCGATGCC ATCCTGCACA CTCCGGGTG	120
CGTCCCTTGC GTTCGTGAGG GCAACGCCTC GAGGTGTTGG GTGGCGATGA CCCCTACGGT	180
GGCCACCAGG GATGGCAAAC TCCCCGCGAC GCAGCTTCGA CGTCACATCG ATCTGCTTGT	240
CGGGAGCGCC ACCCTCTGTT CGGCCCTCTA CGTGGGG	277

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 277 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CATCCCAGCT TCCGCTTACG AGGTGCGCAA CGTGTCCGGG ATATACCATG TCACGAACGA	60
CTGCTCCAAC TCAAGTATTG TGTATGAGGC AGCGGACATG ATCATGCACA CCCCCGGGTG	120
CGTCCCTTGC GTCCGGGAGA GTAATTCTC CCGTTGCTGG GTAGCGCTCA CTCCACGCT	180
CGCGGCCAGG AACAGCAGCA TCCCCACAC GACAATACGA CGCCACGTCG ATTTGCTCGT	240
TGGGGCGGCT GCTCTCTGTT CCGCTATGTA CGTTGGG	277

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 277 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CACCCCGGTC TCCGCTGCCG AAGTGAAGAA CATCAGTACC GGCTACATGG TGACCAACGA	60
CTGCACCAAT GATAGCATT CCTGGCAACT CCAGGCTGCT GTCCCTCCACG TCCCCGGGTG	120
CGTCCCGTGC GAGAAAGTGG GGAATACATC TCGGTGCTGG ATACCGGTCT CACCGAATGT	180
GGCCGTGCAG CAGCCCGGCG CCCTCACGCA GGGCTTACGG ACGCACATTG ACATGGTTGT	240

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GATGTCGGCC ACGCTCTGCT CCGCTCTTA CGTGGGG

277

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 277 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AGTGCCAGTG TCTGCAGTGG AAGTCAGGAA CATTAGTTCT AGCTACTACG CCACTAATGA	60
TTGCTCAAAC AACAGCATCA CCTGGCAGCT CACTGACGCA GTTCTCCATC TTCCCTGGATG	120
CGTCCCATGT GAGAAATGATA ATGGCACCTT GCATTGCTGG ATACAAGTAA CACCCAACGT	180
GGCTGTGAAA CACCGCGGTG CGCTCACTCG TAGCCTGCGA ACACACGTCG ACATGATCGT	240
AATGGCAGCT ACCGGCTTGCT CGGGCTTGTGTA TGTGGGA	277

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGTA CAGCCTCCAG GCCCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAGACT	120
GGGTCCCTTTC TTGGATAAAC CCACTCTATG CCCGGTCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGTA CAGCCTCCAG GCCCCCCCCT	60
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CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTAC CGGAAAGACT	120
GGGTCCCTTC TTGGATAAAC CCACTCTATG TCCGGTCATT TGGGCACGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GCAGAAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGCGTG CAGCCTCCAG GACCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CAGGACGACC	120
GGGTCCCTTC TTGGATCAAC CCGCTCAATG CCTGGAGATT TGGGCGTGCC CCCGCGAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCAGAAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGCGTG CAGCCTCCAG GACCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CAGGACGACC	120
GGGTCCCTTC TTGGATCAAC CCGCTCAATG CCTGGAGATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

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GC GGAAAGCG CCTAGCCATG GCGTTAGTAC GAGTGTGCGTG CAGCCTCCAG GACCCCCCCT	60
CCCCGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATCGC TGGGGTGACC	120
GGGTCTTTTC TTGGAGCAAC CCGCTCAATA CCCAGAAATT TGGGCGTGCC CCCGCGAGAT	180
CACTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGCGTA CAGCCTCCAG GCCCCCCCCCT	60
CCCCGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAAGACT	120
GGGTCTTTTC TTGGATAAAC CCACTCTATG CCCGGCCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGCGTA CAGCCTCCAG GCCCCCCCCCT	60
CCCCGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAAGACT	120
GGGTCTTTTC TTGGATAAAC CCACTCTATG CCCGGCCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGTA CAGCCTCCAG GCCCCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CAGGAAGACT	120
GGGTCCCTTC TTGGATAAAC CCACTCTATG CCTGGCCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 194 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GCAGAAAGCG TCTAGCCATG GCGTTAGTAT GAGTGTGTA CAGCCTCCAG GTCCCCCCCCCT	60
CCCGGGAGAG CCATAGTGGT CTGCGGAACC GGTGAGTACA CCGGAATTGC CGGGAAAGACT	120
GGGTCCCTTC TTGGATAAAC CCACTCTATG CCCGGCCATT TGGGCGTGCC CCCGCAAGAC	180
TGCTAGCCGA GTAG	194

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What is claimed is:

1. An oligonucleotide consisting of the sequence of Seq ID No. 1.
2. An oligonucleotide consisting of the sequence of Seq ID No. 2.
3. An oligonucleotide consisting of the sequence of Seq ID No. 3.
4. An oligonucleotide consisting of the sequence of Seq ID No. 4.
5. An oligonucleotide consisting of the sequence of Seq ID No. 5.
6. An oligonucleotide consisting of the sequence of Seq ID No. 6.
7. An oligonucleotide consisting of the sequence of Seq ID No. 7.
8. An oligonucleotide consisting of the sequence of Seq ID No. 8.
9. An oligonucleotide consisting of the sequence of Seq ID No. 9.
10. A pair of PCR primers wherein the sense primer consists of Seq ID NO. 1 and the antisense primer is selected from the group consisting of Seq ID NO 2, Seq ID NO. 3, Seq ID NO. 4 and Seq ID NO. 5.
11. A pair of PCR primers wherein the antisense primer consists of Seq ID NO. 6 and the sense primer is selected from the group consisting of Seq ID NO 7, Seq ID NO. 8, and Seq ID NO. 9.
12. A method of determining the HCV genotype of an HCV strain, said method comprising the steps of:
 - (a) subjecting said HCV strain to one or more stages of PCR, wherein the one or more stages of PCR utilizes a sense probe from the core or E1 region of the HCV genome and an antisense probe from the core or E1 region of the HCV genome;
 - (b) forming a heteroduplex by denaturing and reannealing mixtures of the amplified product obtained in step (a) with DNA or RNA fragments of a known HCV genotype;

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(c) comparing the mobility of said heteroduplex on a system that separates by size with the mobility of a homoduplex of the DNA or RNA fragments of known genotype to determine the genotype of the HCV strain.

13. The method of claim 12 wherein said HCV strain is subjected to two stages of PCR, wherein the first set of primers comprise a universal sense probe from the core or E1 regions of the HCV genome and a type specific antisense probe from the core or E1 regions of the HCV genome, and wherein the second set of PCR primers comprise a universal antisense probe from the core or E1 regions of the HCV genome and a type specific sense probe from the core or E1 regions of the HCV genome.

14. The method of claim 12 wherein the first set of PCR primers are those according to claim 10 and wherein the second set of PCR primers are those according to claim 11.

15. The method of claim 12 wherein said DNA or RNA fragments of a known genotype comprise a DNA probe.

16. The method of claim 15 wherein said probe is single stranded.

17. The method of claim 16 wherein said DNA probe is radiolabeled.

18. The method of claim 16 wherein said single stranded DNA probe is obtained by PCR amplification.

19. The method of claim 18 wherein said DNA probe is obtained by two step PCR amplification utilizing the primers of claim 10 for the first step and claim 11 for the second step.

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20. The method of claim 12 wherein said HCV strain is present in an excess in the mixture forming the heteroduplex.

21. A method to predict the response to drug therapy of a strain of HCV from a patient infected with said strain of HCV, said method comprising determining the sensitivity of known HCV genotypes to said drug therapy, determining the HCV genotype of said strain of HCV by the method according to claim 12, and comparing said HCV genotype of said strain prior to said drug therapy with said sensitivity of known HCV genotypes to said drug therapy.

22. A method to predict the response to a therapeutic vaccine of a strain of HCV from a patient infected with said strain of HCV, said method comprising determining the sensitivity of known HCV genotypes to said therapeutic vaccine, determining the HCV genotype of said strain of HCV by the method according to claim 12, and comparing said HCV genotype of said strain prior to administration of said therapeutic vaccine with said sensitivity of known HCV genotypes to said therapeutic vaccine.

23. A method to predict the appropriateness of a prophylactic vaccine composition for a given sample population said method comprising determining the genotype of said prophylactic vaccine, determining the predominance of known HCV genotypes in said sample population by the method according to claim 12, and comparing said HCV genotype of said prophylactic vaccine strain to the determined predominant genotype prior to administration of said prophylactic vaccine to said population sample.

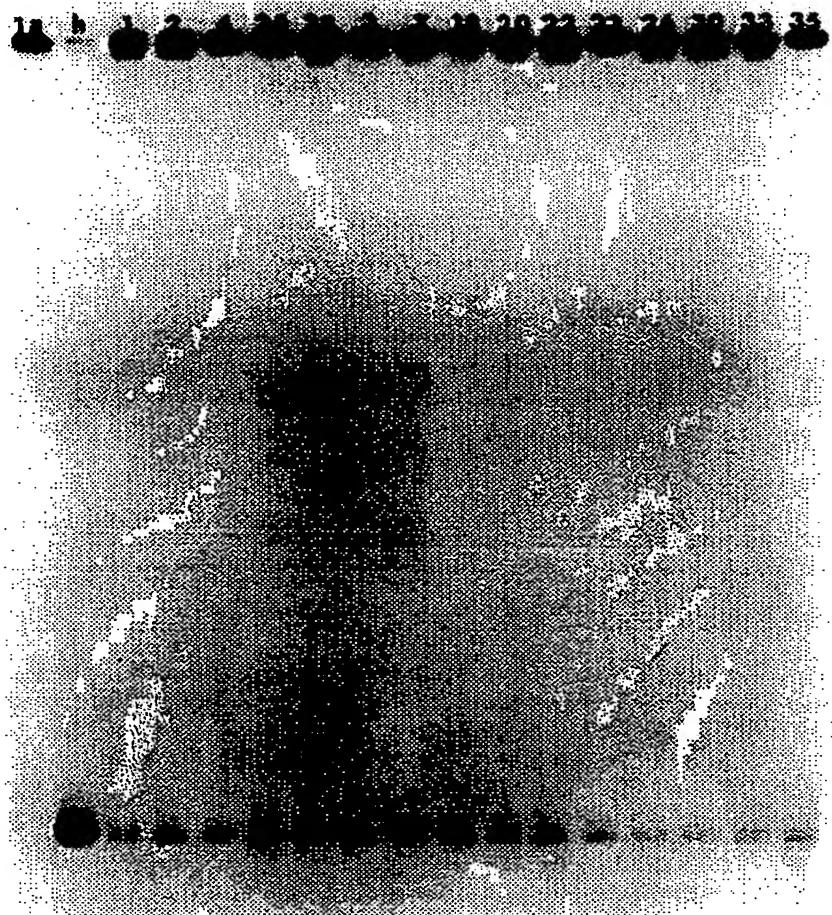


FIG. 1A

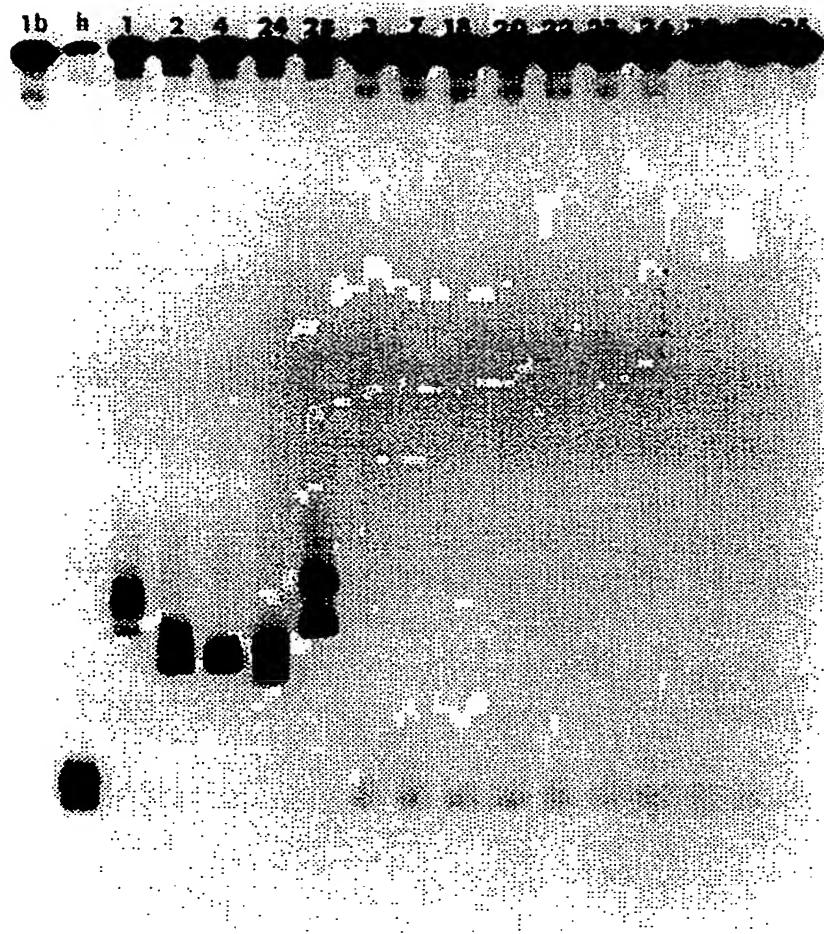


FIG. 1B

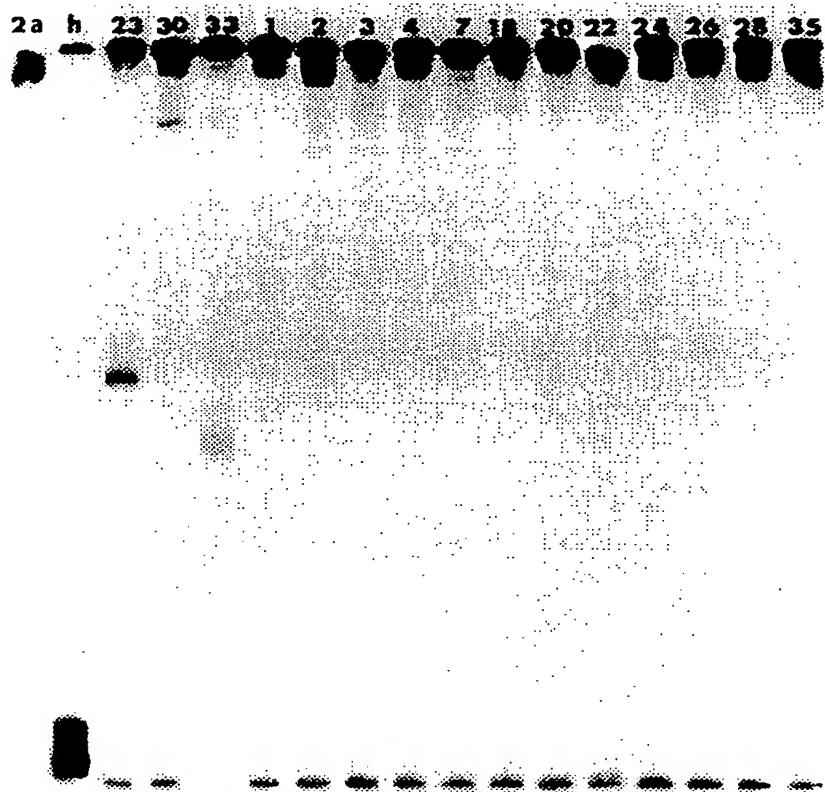


FIG. 1C

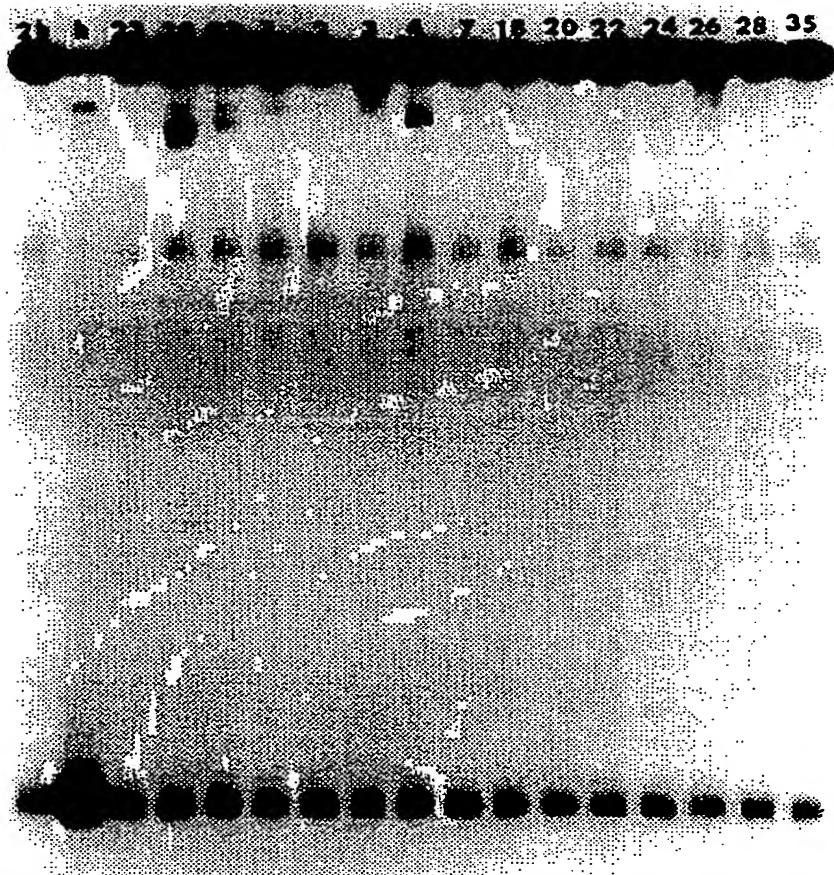


FIG. 1D

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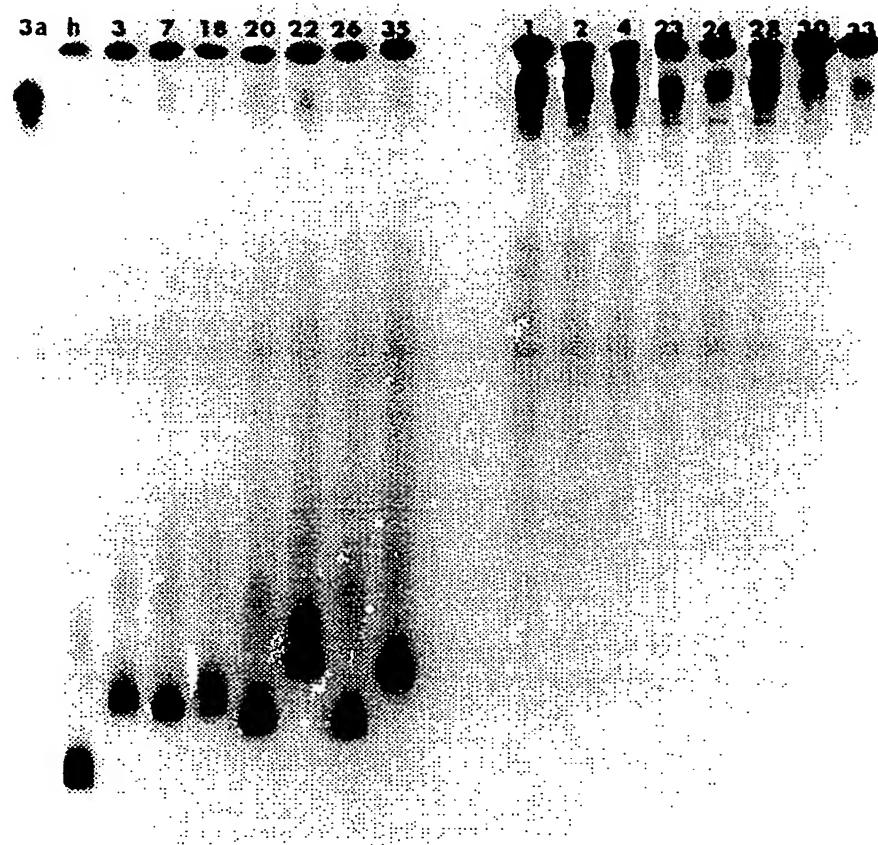


FIG. 1E

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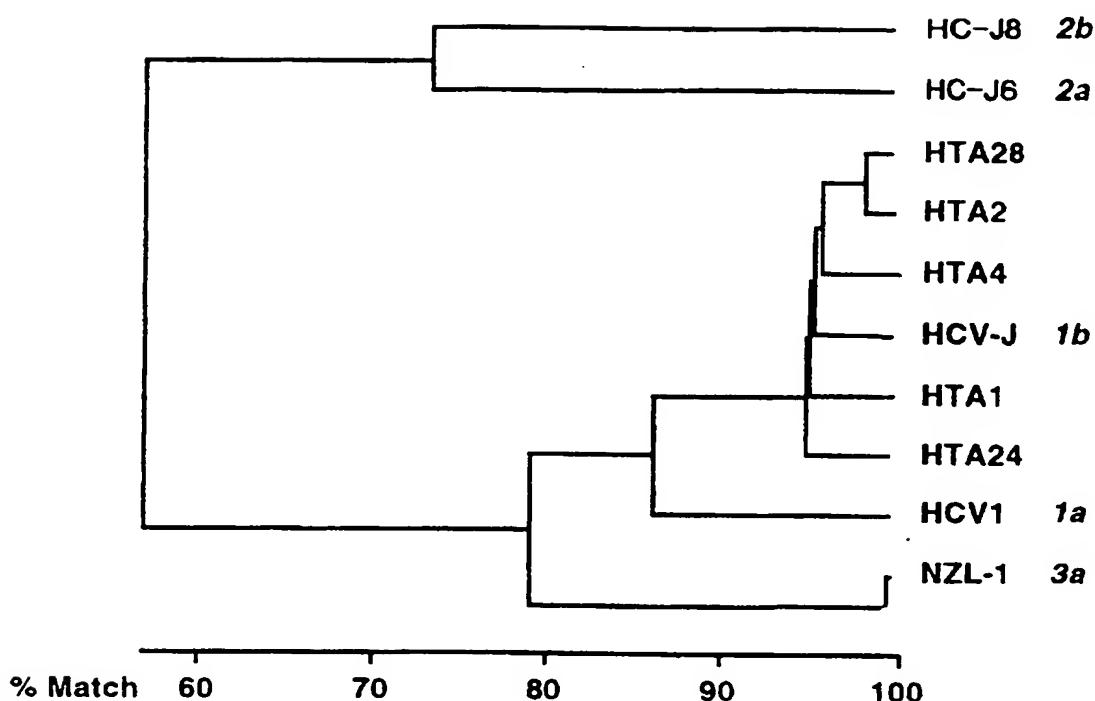


FIG. 2A

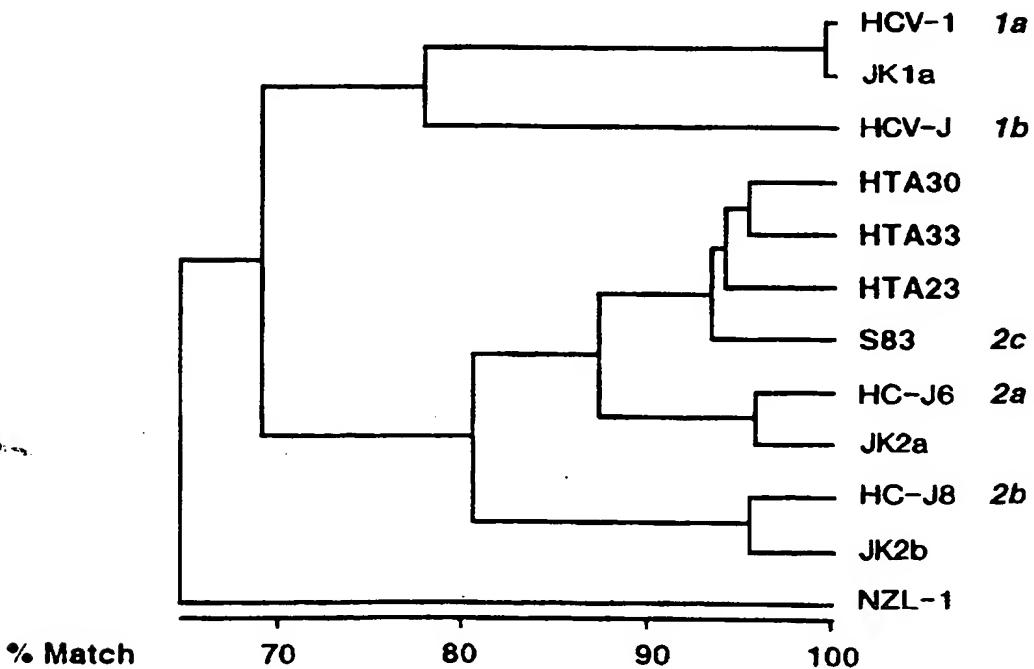


FIG. 2B

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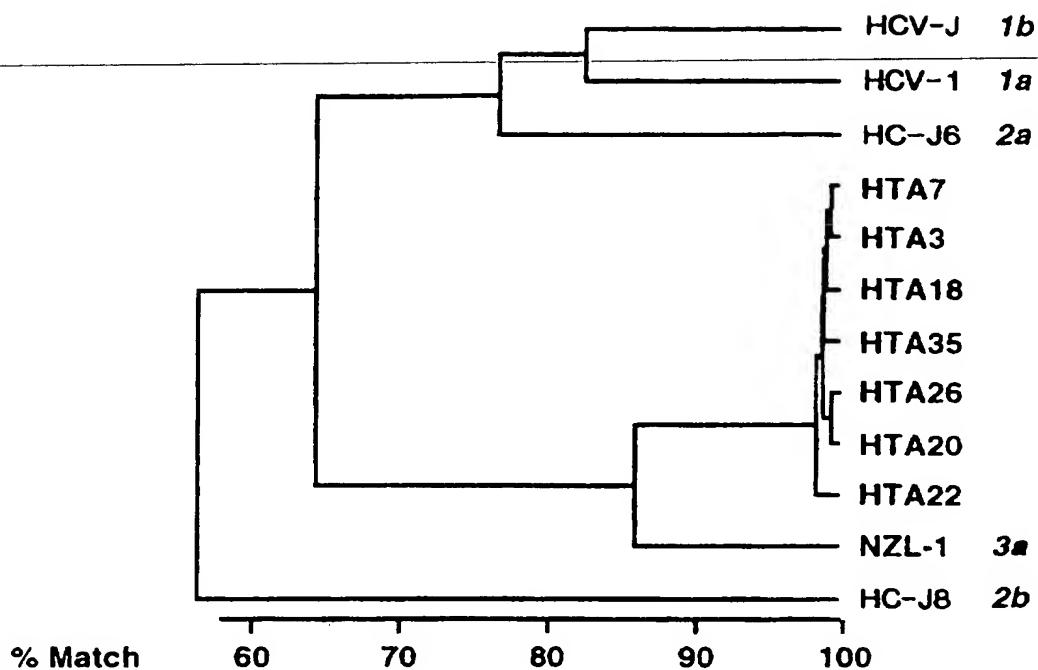


FIG. 2C

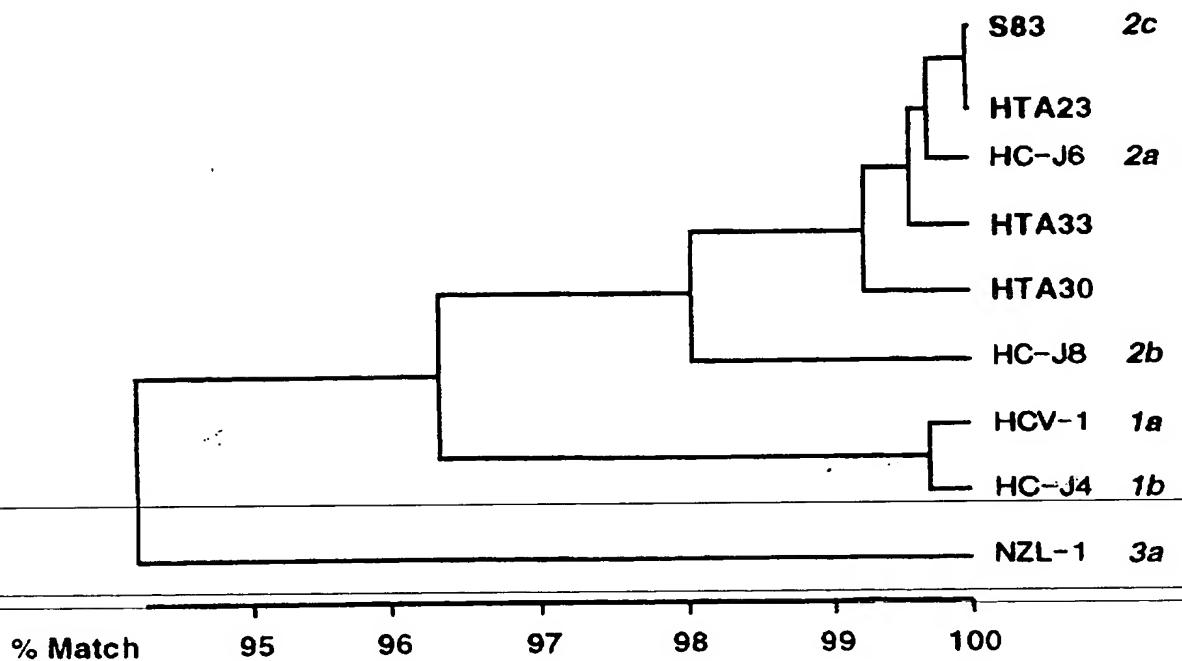


FIG. 2D

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HTA1	AACTCAAGCATTGTGTATGAAGCGGCGGACATGATCATGCACACCCCCGGGTGCGTGCCA
HTA2	AACTCAAGCATTGTGTATGAGGCAGCGGAAGTGTATCATGCACATTCCCGGGTGCCTGCC
HTA4	AACTCAAGCATAGTATATGAGGCAGCGGAACATAATCATGCATACCCCCGGGTGCGTGCC
HTA24	AAACAGGACATTGTGTATGAGGCAGCGGAACCTGTATCATGCACGTCCCCGGGTGCGTGCC
HTA28	AACTCAAGCATCGTGTATGAGGCAGCGGAAGTGTATCATGCACATTCCCTGGGTGCGTGCC
HCV-1	AACTCGAGTATTGTGTACGAGGCAGCGGAACATGCCATCTGCACACTCCGGGTGCGTCCCT
HCV-J	AACTCAAGTATTGTGTATGAGGCAGCGGAACATGATCATGCACACCCCCGGGTGCGTGCC
HC-J6	AATGATAGCATTACCTGGCAACTCCAGGCTGCTGCTCCACGTCCCCGGGTGCGTCCCC
HC-J8	AACAAACAGCATCACCTGGCAGCTACTGACGCAGTTCTCCATCTTCCATGGATGCGTCCCC
NZL-1	AATAGCAGTATTGTGTATGAGGCCATGATGTCATTCTGCACACACCCGGTGTGTACCT

HTA1	TGCGTCCGGGAGGGCAATCTCTCCCGCTGCTGGGTAGCGCTCACTCCCACGCTCGCGGCC
HTA2	TGCGTTCGGGAGAGCAATCTCTCCCGCTGCTGGGTAGCGCTCACCCCCACACTCGCGGCC
HTA4	TGTGTTCGGGAGGTCAACTCTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCGGCC
HTA24	TGCGTTCGGGAGGGCAACTCTCCCGATGCTGGGTAGCGCTCACTCCCACGATCGCGGCC
HTA28	TGCGTTCGGGAGGGCGACTTCTCCCGCTGCTGGGTAGCGCTCACCCCCACACTCGCGGCC
HCV-1	TGCGTTCGTGAGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACC
HCV-J	TGCGTCCGGGAGAGTAATTCTCCCGTTGCTGGGTAGCGCTCACTCCCACGCTCGCGGCC
HC-J6	TGCGAGAAAGTGGGAATACATCTCGGTGCTGGATACCGGTCTCACCGAATGTGGCGTG
HC-J8	TGTGAGAATGATAATGGCACCTTGATGCTGGATACAAGTAACACCCAACGTGGCTGTG
NZL-1	TGTGTCCAGGACGGCAATACATCTACGTGCTGGACCCAGTGACACACTACAGTGGCAGTC
JK1a	TGTGTTCGCGAGGGCAACGCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACC

HTA1	AGAAAACAGCAGCGTTCTACTACGACAATACGACGCCATGTCACTGCTAGTAGGAGCG
HTA2	AGGAACAGCAGCGTCCCACCGACAATACGACGCCACGTCGACTTGCTCGTTGGGGCG
HTA4	AGGAACACTCCAGCGTCCCACGACAATACGACGCCACGTCGACTTGCTCGTTGGGGCG
HTA24	AGGAACAGCAGTGTCCCCTACGACCAATACGACGCCACGTCGACTTGCTCGTTGGGGCG
HTA28	AGGAATAACAGCGTCCCACGACAATACGACGCCACGTCGACTTGCTCGTTGGGGCG
HCV-1	AGGGATGGCAAACCTCCCGCGACGAGCTTCGACGTACATCGATCTGCTTGCGGGAGC
HCV-J	AGGAACAGCAGCATCCCCACACGACAATACGACGCCACGTCGATTGCTCGTTGGGGCG
HC-J6	CAGCAGCCCGGCGCCCTCACCGAGGGCTTACGGACGCACATTGACATGGTTGTATGTCC
HC-J8	AAACACCGCGGTGCGCTCACTCGTAGCCTGCGAACACACGTGACATGATCGTAATGGCA
NZL-1	AGGTACGTCCGGAGCAACTACTGCTTCGATACGCACTACGAGTCATGTGGACCTATTAGTAGGCGC

HTA1	GCTGCTTTTGCTCCGCCATGTACGTGGGGACCTCTGCGGATCTATTTCCTCGTCTCC
HTA2	GCTGCCTCTGCTCCGCTATGTATGTGGGGATCTCTGCGGATCTGTTTCCTGCTCTCC
HTA4	GCTGCTTTCTGCTCCGCTATGTACGTGGGGATCTATGCGGATCTGTTCTACTTGTCTCT
HTA24	GCTGCTCTTGCTCCGCTATGTACGTGGGGATCTCTGCGGATCTGCTCTTCCTGCTCTCC
HTA28	GCTGCTCTCTGCTCCGCTATGTACGTGGGGATCTCTGCGGATCTGTTTCCTGCTCTCC
HCV-1	GCCACCCCTCTGTTCCGCTTACGTGGGGACCTATGCGGGTCTGCTTTCTGCGGCC
HCV-J	GCTGCTCTGTTCCGCTATGTACGTGGGGATCTCTGCGGATCCGTTTTCTCGTCTCC
HC-J6	GCCACGCTCTGCTCCGCTTACGTGGGGACCTCTGCGGTGGGGTATGCTTGCGGCC
HC-J8	GCTACGGCTGCTCGCCCTGTATGTGGAGATGTGCGGGCCGTATGATTCTATCG
NZL-1	GCCACGATGTGCTCTGCGCTACGTGGGTGATATGTGTGGGCTGCTTTCTCGTGGGA

HTA1	CAACTGTTCACCTTCTGCCCGCCGGCATACAGTACAGGAACGTGCAATTGCTCGATC
HTA2	CAACTGTTCACCTTCTGCCCTGCCGGCATGAGACAGTACAGGAACGTGCAATTGTTCAATC
HTA4	CAGCTGTTCACCTTCTACCTCGCCGGACGAGACAGTGCAGGAACGTGCAATTGTTCAATC
HTA24	CAGTTGTTCACTTCTCGCCTGCCAGCATCAGACGGTACAGGAACGTGCAACTGCTCAATC
HTA28	CAACTGTTCACCTTCTGCCGGCATGCGACAGTACAGGAACGTGCAATTGTTCAATC

FIG. 3A-1

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HCV-1	CAACTGTTCACCTTCTCTCCCAGGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATC
HCV-J	CAGCTGTTCACCTTCTCACCTCGCCGGTATGAGACGGTACAAGATTGCAATTGCTCAATC
HC-J6	CAGATGTTCATTGTCTGCCACAGCACCAACTGGTTGTGCAAGACTGCAATTGCTCCATC
HC-J8	CAGGCTTCATGGTATCACCACAACGCCACAACCTCACCCAAAGAGTGCAACTGTTCCATC
NZL-1	CAAAGCCTTCACGTTCAGACCTCGACGCCATCAAACGGTCCAGACCTGTAACTGCTCGCTG

HTA1	TATCCC
HTA2	TATCCC
HTA4	TATCCC
HTA24	TATCCC
HTA28	TATCCC
HCV-1	TATCCC
HCV-J	TATCCC
HC-J6	TACCCCT
HC-J8	TACCAA
NZL-1	TACCCA

FIG. 3A-2

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JK1a	CGCAACTCCACGGGGCTTACACGTCACCAATGATTGCCCTAACCTGAGTATTGTGTAC
JK2a	AAGAACACCAGCGACAGCTACATGGTACCAATGACTGCCAAAATGACAGCATCACCTGG
JK2b	AGGAACATCAGTTCTAGCTACTACGCCACTAATGACTGCTCGAACACAGCATCACCTGG
SW83 . 2c	AAGGACACCAGCGACTCCTACATGCCACCAACGATTGCTCAACTCTAGTATCGTTGG
HTA23	AAAAAACACCAGCATCTCCTATATGGCGACCAACGACTGCTCAACTCCAGCATCGTTGG
HTA33	AAGAACACCAGCGACTCCTACATGGCGACTAACGACTGCTCTAACCTCAGCATCGTTGG
HTA30	AAGAACACCAGCACCTCCTACATGGTACCTAACGATTGCTCAACTCCAGCATCGTTGG
HCV-1	CGCAACTCCACGGGGCTTACACGTCACCAATGATTGCCCTAACCTGAGTATTGTGTAC
HCV-J	CGCAACGTGTCCGGGATATACCATGTCACGAACGACTGCTCAACTCAAGTATTGTGTAT
HC-J8	AGGAACATTAGTTCTAGCTACTACGCCACTAACGATTGCTCAAACAAACAGCATCACCTGG
NZL-1	CGGAATACGTCTGGCCTACGTCTAACGACTGTTCAAATAGCAGTATTGTGTAT
HC-J6	AAGAACATCAGTACCGGCTACATGGTACCAACGACTGACCAATAGCAGTATTGTGTAT

* * * * *

JK1a	GAGACGGCCGATGCCATCCTGCACACTCCGGGGTGCCTCCCTGTGTTCGCGAGGGCAAC
JK2a	CAGCTTGAGGCTCGGGTCTCCACGTCACCTCCGGTGCCTCCGTGCGAGAGAGTGGAAAT
JK2b	CAGCTCACCAACCGCAGTTCTCCACCTCCGGATGCGTCCCATGTGAGAATAATAATGGC
SW83 . 2c	CAGCTTGAGGAGCAGTGCTCCATACTCCTGGATGCGTCCCTTGAGCGTACCGCAAC
HTA23	CAGTTTGACGGCGCAGTGCTCCATACTCCTGGATGCGTCCCTTGCGAACGGACGGCAAC
HTA33	CAGCTTGAGGACGCAGTGCTCCATGTCCCTGGATGCGTCCCTTGAGAGACTGGCAAT
HTA30	CAACTTGAGGCGCAGTGCTCCATGTTCCCTGGATGCGTCCCTTGAGCAGATCGGCAAC
HCV-1	GAGGGGGCGATGCCATCCTGCACACTCCGGGTGCCTCCCTGCGTTCTGAGGGCAAC
HCV-J	GAGGCAGCGGACATGATCATGCACACCCCCGGGTGCCTGCCCTGCGTCCGGAGAGTAAT
HC-J8	CAGCTCACTGACGCAGTTCTCCATCTCCTGGATGCGTCCCATGTGAGAATGATAATGGC
NZL-1	GAGGCCGATGATGTCATTCTGCACACACCCGGCTGTACCTTGTGTCAGGACGGCAAT
HC-J6	CAACTCCAGGCTGCTGTCCTCACGTCCCCGGGTGCCTGCCGTGCGAGAAAGTGGGAAT

* * * * *

JK1a	GCCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCACCAGGGATGGCAAACCTCCCC
JK2a	ACATCTCGGTGCTGGATACCGGTCTCACCAAACGTGGCTGCGGAGCCGGCGCCCTC
JK2b	ACCTTGCAATTGCTGGATACAAGTAACACCTAATGTGGCCATGGAAACATCGCGGCGCACTC
SW83 . 2c	GTCTCTCGATGTTGGGTGCCGGTTGCCCTAACCTCGCCATAAGTCACCTGGCGCTCTC
HTA23	GCGTCCCGGTTGGGTGCCGGTTGCCCTAACCTGGCTATAAGCAACCCGGCGCCCTC
HTA33	ACGTCTCGGTGCTGGGTGCCGGTTACCCCAATGTGGCTACAAGTCACCCGGCGCTCTC
HTA30	GTGTCTCAGTGGTGGGTGCCGGTTACCCCAATATGGCCATAAGTACACCCGGCGCTCTC
HCV-1	GCCTCGAGGTGTTGGGTGCCGATGACCCCTACGGTGGCACCGGGATGGCAAACCTCCCC
HCV-J	TTCTCCCGGTTGCTGGTAGCGCTACTCCCACGCTCGCGGCCAGGAACAGCAGCATCCCC
HC-J8	ACCTTGCAATTGCTGGATACAAGTAACACCCACGTGGCTGTGAAACACCGCGGTGCGCTC
NZL-1	ACATCTACGTGCTGGACCCAGTGACACCTACAGTGGCAGTCAGGTACGTGGAGCAACT
HC-J6	ACATCTCGGTGCTGGATACCGGTCTCACCGAATGTGGCGTGCAGCACGGCGCCCTC

* * * * *

JK1a	GCGACGCAGCTCGACGTACATCGATCTGCTTGTGGAGCGCCACCCCTGTTGGCC
JK2a	ACCGAGGGCTTGCAGCGACATCGACATGATTGTGATGTCGGCCACGCTCTGCTCCGCT
JK2b	ACTCACAACTCGGGACACATGTCGACATGATCGTAATGGCAGCTACGGCTGTTGGCC
SW83 . 2c	ACTAAGGGCTCGGAGCACACATCGATATCATCGTATGCTGCTACGGCTGTTCTGCC
HTA23	ACTAAGGGCATACGAACGCACATTGATGTCATCGTAATGTCGCTACGCTGTTCTGCC
HTA33	ACCAGGGCTTGCAGCGACATCGATGTCATCGTATGCTGAGCCACGCTGCTCCGCT
HTA30	ACTAAGGGCTTGCAGCGACATCGACGGCATCGTATGCTGCTACGCTGTTCTGCC
HCV-1	GCGACGCAGCTCGACGTACATCGATCTGCTTGTGGAGCGCCACCCCTGTTGGCC
HCV-J	ACCACGACAATACGACGCCACGTCGATTGCTCGTTGGGGCGCTGCTCTGTTCCGCT
HC-J8	ACTCGTAGGCTCGAACACACGTCGACATGATCGTAATGGCAGCTACGGCTGCTGGCC
NZL-1	ACTGCTTCGATACGAGTCATGTGGACCTATTAGTAGGCGGGCCACGATGTGCTCTGCG
HC-J6	ACGCAGGGCTTACGGACGCACATTGACATGGTTGTGATGTCGGCCACGCTGCTCCGCT

* * * * *

JK1a CTCTACGTGGGGATCTGTGGGGCTGTCTTCTGTGGCCAACTGTTACCTTCTCT

FIG. 3B-1

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JK2a	CTCTACGTGGGGGACCTCTGTGGCGGGATGATGCTCGCAGCCCAGATGTTCATCGTTCTG
JK2b	TTGTACGTAGGAGACGTGTGTGGGGCTGTGATGATTGTGTCTCAGGCCCTATAATATCA
SW83.2c	CTTTATGTGGGGGACGTGTGTGGCGCCTGATGCTGGCCCTCAGGTCGTCGTGTCG
HTA23	CTTTACGTGGGGGACGTGTGTGGCGCTGATGATTGCCCTCAGGTCGTCATTGTGTC
HTA33	CTCTATGTGGGGGACGTGTGTGGCGCGTTGACGATAGCCGCTCAGGTTGTCATCGTATCG
HTA30	CTTTATGTGGGGGACGTGTGTGGCGCGTTGATGATAGCCGCCAGGTCGTCATCGTATCG
HCV-1	CTCTACGTGGGGGACCTATGCGGGCTGTCTTCTTGTCCGCAACTGTTCACCTTCTCT
HCV-J	ATGTACGTGGGGATCTCTGCGGATCCGTTTCTCGTCTCCAGCTGTTCACCTTCTCA
HC-J8	TTGTATGTGGGAGATGTGTGCGGGCCGTGATGATTCTATCGCAGGCTTCATGGTATCA
NZL-1	CTCTACGTGGGTGATATGTGTGGGGCTGTCTTCTCGTGGGACAAGCCTCACGTTCAGA
HC-J6	CTTTACGTGGGGGACCTCTGCGGTGGGGTGTGCTGCAGCCCAGATGTTCATGTCTCG
* * * * *	
JK1a	CCCAAGCGCCACTGGACGACGCAAGGTTGCAAT
JK2a	CCCGAGAACCACTGGTTCTGCGAGGAATGCAAT
JK2b	CCAGAACACCCATAACTTCACCCAAGAGTGCAAC
SW83.2c	CCAACACCCATACGTTGTCCAGGAATGCAAC
HTA23	CCGCAGCATTACCACTTGTCCAGGACTGCAAT
HTA33	CCACGGCACCACCACCTTGTCCAGGACTGCAAT
HTA30	CCACAGCACCACCACCTTGTCCACGACTGCAAC
HCV-1	CCCAGGCGCCACTGGACGACGCAAGGTTGCAAT
HCV-J	CCTCGCCGGTATGAGACGGTACAAGATTGCAAT
HC-J8	CCACAACGCCACAACCTCACCCAAGAGTGCAAC
NZL-1	CCTCGACGCCATCAAACGGTCCAGACCTGTAAC
HC-J6	CCACAGCACCACCTGGTTGTGCAAGACTGCAAT
* * * * *	

FIG. 3B-2

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HTA3	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTTACCAACG
HTA7	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTTACCAACG
HTA18	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTTACCAACG
HTA20	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTTACCAACG
HTA22	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTTACCAACG
HTA26	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTTACCAACG
HTA35	TCATCCAACATCTAGTCTAGAGTGGCGGAATACGTCTGGCCTCTATGTCTCACCAACG
NZL-1	TCATCCAGCAGCCAGTCTAGAGTGGCGGAATACGTCTGGCCTCTACGTCTTACCAACG
HCV-1	TGTGCCGCTTCGGCTTACCAAGTGCAGCTCCACGGGGCTTACCAACGTCACCAATGA
HCV-J	CATCCCAGCTTCCGCTTACGAGGTGCGAACGTGTCCGGATATACCATGTCACGAACG
HC-J6	CACCCCCGGTCTCCGCTGCCGAAGTGAAGAACATCAGTACCGGCTACATGGTACCAACG
HC-J8	AGTGCCAGTGTCTGCAGTGGAAAGTCAGGAACATTAGTTCTAGCTACTACGCCACTAATGA

HTA3	CTGTTCCAATAACATTATTGTGTATGAGGCCGATGACGTATCCTGCACACGGGGCTG
HTA7	CTGTTCCAATAACATCATTGTGTATGAGGCCGATGACGTATCCTGCAGCACCGGGCTG
HTA18	CTGTTCCAATAATATTATTGTGTATGAGGCCGACGACGTATCCTGCAGCCCCGGCTG
HTA20	CTGTTCCAATAACATTATTGTGTATGAGGCCGATGACGTATCCTGCACACACCGGGCTG
HTA22	CTGTTCCAATAACAGTATTGTGTATGAGGCCGATCACGTATCCTGCACACACCGGGCTG
HTA26	CTGTTCTAATAACATTATTGTGTATGAGGCCGATGACGTATCCTGCACACACCGGGCTG
HTA35	CTGTTCCAACAAACATTATTGTGTATGAGGCCGATGACGTATTCTGCACACACGGGGCTG
NZL-1	CTGTTCCAATAGCAGTATTGTGTATGAGGCCGATGATGTATTCTGCACACACCGGGCTG
HCV-1	TTGCCCTAACTCGAGTATTGTGTACGAGGCCGATGCCATCCTGCACACTCCGGGGTG
HCV-J	CTGCTCCAACCTCAAGTATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGTG
HC-J6	CTGCACCAATGATAGCATACCTGGCAACTCCAGGCTGCTGCTCCACGTCCCCGGTG
HC-J8	TTGCTCAAACAAACAGCAGTACCTGGCAGCTCACTGACGGAGTTCTCCATCTCCTGGATG

HTA3	TGTACCTTGTGTTAGGACGGTAATACATCCAAGTGTGGACCCCAGTGACACCTACAGT
HTA7	TGTACCTTGTGTTAGGACGGCAATACATCCACGTGTGGACCCCAGTGACACCTACAGT
HTA18	TGTACCTTGTGTTAGGACGGCAATACATCCACGTGTGGATCCCAGTGACACCTACAGT
HTA20	TGTACCTTGTGTTAGGACGGCAATACATCCACGTGTGGACCCCAGTGACACCTACAGT
HTA22	TGTACCTTGTGTTCAAGCCAACAATAAATCCAAATGCTGGACCCCAGTGACACCTACAGT
HTA26	TGTACCTTGTGTTAGGACGGCAATGCATCCACGTGTGGACCCCAGTAACACCTACAGT
HTA35	CGTACCTTGTGTACAGGACGGCAATACATCCACGTGTGGACCCCAGTGACACCTACAGT
NZL-1	TGTACCTTGTGTCAGGACGGCAATACATCTACGTGTGGACCCCAGTGACACCTACAGT
HCV-1	CGTCCCTTGCCTCGTGAGGGCAACGCCTCGAGGTGTGGTGGCGATGACCCCTACGGT
HCV-J	CGTGCCTCGCTCCGGGAGAGTAATTCTCCGTTGTGGTAGCGCTCACTCCACGCT
HC-J6	CGTCCCGTGCAGAAAAGTGGGAATACATCTCGGTGTGGATACCGGTCTACCGAATGT
HC-J8	CGTCCCATGTGAGAATGATAATGGCACCTTGCATTGTGGATACAAGTAACACCCAACGT

HTA3	GGCAGTCAGGTACGTCGGAGCAACCACCGCTCAATACGCAGCCACGTGGACCTATTATT
HTA7	GGCAGTCAGGTACGTCGGAGCAACCACCGCTCAATACGCAGCCATGTGGACCTATTAGT
HTA18	GGCAGTCAGGTACGCCGGAGCAACCACCGCTCAATACGCAGCCATGTGGACCTGTAGT
HTA20	ATCAGTCAGGTACGTCGGAGCAACCACCGCTCAATACGCAGCCATGTGGACCTACTATT
HTA22	ATCAGTCGAGTACGTCGGAGCAACCACCGCTCAATACGCAGCCATGTGGACCTACTATT
HTA26	ATCAGTCAGGTACGTCGGAGCAACCACCGCTTCAGTACGCAGCCATGTGGACCTACTATT
HTA35	GGCAGTCAGGTACGTCGGAGCAACTACCGCTCAATACGCAGCCATGTGGACCTATTATT
NZL-1	GGCAGTCAGGTACGTCGGAGCAACTACTGCTTCGATACGCAGTCATGTGGACCTATTAGT
HCV-1	GGCCACCAGGGATGGCAAACCTCCCCCGGACGCGAGCTTCGACGTCACATCGATCTGCTTGT
HCV-J	CGCGGCCAGGAAACAGCAGCATTCCCCACCAACGACAATACGACGCCACGTCATTGCTCGT
HC-J6	GGCCGTGCAGCAGCAGCCGGGCCCTCACGCAGGGCTTACGGACGCACATTGACATGGTTGT
HC-J8	GGCTGTGAAACACCGCGGTTGCGCTACTCGTAGCCTGCGAACACACGTCGACATGATCGT

HTA3 GGGCGCGGCCACGATGTGCTCTGCGCTCTACGTGGGT

FIG. 3C-1

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HTA 7	GGGCGCGGCCACGATGTGCTCTGCCTCTACGTGGGT
HTA18	GGGCGCGGCCACGATGTGCTCTGCCTCTACGTGGGT
HTA20	GGGCGCGGCCACGATGTGCTCTGCCTCTACGTGGGT
HTA22	GGGCGCGGCCACGATGTGCTCTGCCTCTACGTGGGT
HTA26	GGGCGCGGCCACGATGTGCTCTGCCTCTATGTGGGT
HTA35	GGGCGCGGCCACGATGTGCTCTGCCTCTACGTGGGT
NZL-1	AGGCGCGGCCACGATGTGCTCTGCCTCTACGTGGGT
HCV-1	CGGGAGCGCCACCCCTCTGTTGGCCCTCTACGTGGGG
HCV-J	TGGGGCGGCTGCTCTGTTCCGCTATGTACGTTGGG
HC-J6	GATGTCCGCCACGCTCTGCTCCGCTCTTACGTGGGG
HC-J8	AATGGCAGCTACGGCCTGCTCGGCCTTGTATGTGGGA

*** * *** * *** * *** * *** * ***

FIG. 3C-2

HC-J6	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGACAGCCTCCAGGCCCCCCCC
HC-J8	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGACAGCCTCCAGGCCCCCCCC
HC-J4	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGAGCCTCCAGGACCCCCCCC
HCV-1	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGAGCCTCCAGGACCCCCCCC
NZL1	-GCGGAAAGCGCTAGCCATGGCGTTAGTACGAGTGTGTCGAGCCTCCAGGACCCCCCCC
S83	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGACAGCCTCCAGGCCCCCCCC
HTA23	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGACAGCCTCCAGGCCCCCCCC
HTA30	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGACAGCCTCCAGGCCCCCCCC
HTA33	-GCAGAAAGCGTCTAGCCATGGCGTTAGTATGAGTGTGTCGACAGCCTCCAGGCCCCCCCC

HC-J6	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAAGAC
HC-J8	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC
HC-J4	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC
HCV-1	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGAC
NZL1	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATCGCTGGGTGAC
S83	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAAGAC
HTA23	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGAAAGAC
HTA30	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGAAAGAC
HTA33	TCCCGGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCGGAAAGAC

HC-J6	TGGGTCTTTCTTGGATAAACCCACTCTATGCCCGTCATTGGCGTGCCCCCGCAAGA
HC-J8	TGGGTCTTTCTTGGATAAACCCACTCTATGCCCGTCATTGGCAGCAGCCCCCGCAAGA
HC-J4	CGGGTCCTTCTTGGATCAACCCGCTCAATGCCCTGGAGATTGGCGTGCCCCCGGAGA
HCV-1	CGGGTCCTTCTTGGATCAACCCGCTCAATGCCCTGGAGATTGGCGTGCCCCCGGAGA
NZL1	CGGGTCCTTCTTGGAGCAACCCGCTCAATACCCAGAAATTGGCGTGCCCCCGGAGA
S83	TGGGTCTTTCTTGGATAAACCCACTCTATGCCCGGCATTGGCGTGCCCCCGCAAGA
HTA23	TGGGTCTTTCTTGGATAAACCCACTCTATGCCCTGGCATTGGCGTGCCCCCGCAAGA
HTA30	TGGGTCTTTCTTGGATAAACCCACTCTATGCCCTGGCATTGGCGTGCCCCCGCAAGA
HTA33	TGGGTCTTTCTTGGATAAACCCACTCTATGCCCGGCATTGGCGTGCCCCCGCAAGA

HC-J6	CTGCTAGCCGAGTAG
HC-J8	CTGCTAGCCGAGTAG
HC-J4	CTGCTAGCCGAGTAG
HCV-1	CTGCTAGCCGAGTAG
NZL1	TCACTAGCCGAGTAG
S83	CTGCTAGCCGAGTAG
HTA23	CTGCTAGCCGAGTAG
HTA30	CTGCTAGCCGAGTAG
HTA33	CTGCTAGCCGAGTAG

FIG. 3D

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/US 97/06062

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 C12Q1/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	HEPATOLOGY, vol. 20, no. 4, - October 1994 page 244a XP002035908 MURASHIMA S. ET AL.,: "Analysis of HCV genome population by PCR heteroduplex method" see the whole document ---	12-23
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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1

Date of the actual completion of the international search

24 July 1997

Date of mailing of the international search report

07.08.97

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Müller, F

INTERNATIONAL SEARCH REPORT

International Application No
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X	WO 92 19743 A (CHIRON CORP) 12 November 1992 see page 125, line 10 ---	2
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